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STABILITY OF NUCLEAR POLYHEDROSIS VIRUS OF THE ARMYWORM *MYTHIMNA SEPARATA* (LEPIDOPTERA : NOCTUIDAE) IN SOIL

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(Received 28 June 1980)

Stability of nuclear polyhedrosis virus (NPV) of the armyworm *Mythimna separata* in a black soil maintained dry or moist and kept outdoors or in darkness for 1, 4, 12, 28 or 64 weeks is investigated. The results of larval mortality due to NPV maintained in the soil indicated that little or no loss of virus infectivity was observed up to 28 or 64 weeks. The incubation period and mean time for total larval death among different treatments did not differ from those of controls. Pupal mortality due to polyhedrosis was very low except for few treatments. It is, therefore, obvious from these findings that *M. separata* NPV remains stable for long periods in soil, and would probably assist in long-term control of the pest.

(Key words: nuclear polyhedrosis virus, *Mythimna separata*, armyworm, virus infectivity, larval mortality, pupal mortality, virus stability in soil)

INTRODUCTION

The armyworm *Mythimna separata* is a serious pest of cereal crops during both *kharif* and *rabi* seasons in India, other Asian countries, Australia, New Zealand and the Pacific Islands (communication from the Commonwealth Institute of Entomology, London). NEELGUND & MATHAD (1972) reported that this pest suffers from a nuclear polyhedrosis. NEELGUND (1975) further suggested that the NPV can be employed for successful biological control of its host.

Generally, insect larvae when die of either nuclear polyhedrosis or granulosis liquefy and release virus material on foliage of host plants. A major portion of this will drop directly on to the soil leaving behind slight virus deposits on foliage which may soon be inactivated by sunlight, temperature and other physical factors. Stability of virus material that reaches the soil is of considerable practical importance since the latter forms chemically and microbiologically a complex environment and would eventually influence virus survival. Reported herein are the results of studies made on the stability of *M. separata* NPV when maintained in soil.

MATERIALS AND METHODS

Black soil (pH 7.7—8 and electrical conductivity 0.25 mmoh/cm) collected from a locality in the Agricultural College Farm, Dharwad Campus, was used. The soil was first ground well using pestle and mortor and the coarse particles were removed. The finely ground soil was then divided into 4 parts of 1,000 g each and to each part 5 ml unpurified polyhedral inclusion body (PIB) suspension (7.3×10^9 PIB/ml) was added and thoroughly mixed in a trough. The mixtures were then transferred separately to 4

small earthen pots which were subsequently maintained as follows: Pot 1. Moistened soil kept in darkness at 26±1°C and covered with brownpaper. Pot 2. Dry soil kept in darkness at 26±1°C and covered with brownpaper. Pot 3. Moistened soil kept outdoors and uncovered. Pot 4. Dry soil kept outdoors and uncovered. Distilled water was added at weekly intervals to Pot 1 and daily to Pot 3. The soil (dry) in Pot 4 was occasionally moistened by rain. The maximum temperature received by the outdoor soil was around 50°C.

For bioassay, 25 g dry/dried soil (the moistened ones were air-dried for 24 hr in darkness at 26 ± 1°C) from each pot was taken separately at intervals of 1, 4, 12, 28 or 64 weeks from the start of the experiment in sterilized beakers (100 ml). To each of these was added 30 ml sterile distilled water and the mixtures were then stirred thoroughly with glass rods. Such mixtures were then kept undisturbed for an hour in darkness or low intensity fluorescent light at 26 ± 1°C in order to enable the heavier and coarser soil particles to settle. Later, about 20 ml supernatant from each sample was withdrawn and stored at 0 or 4°C until it was bioassayed. The pH of the supernatant from the samples receiving different treatments was around neutral. Disease-free, late third instar (8—9 day-old) armyworm larvae selected from the laboratory culture were used for experimentation. Altogether 0.03 ml supernatant—0.01 ml/larva/day—was fed through the formaldehyde-free artificial diet (NEELGUND & MATHAD, 1974) to the larvae. Two groups of control larvae, fed on (a) supernatant from the soil which was not treated with the virus but kept dry in darkness at 26±1°C and (b) distilled water, were maintained. Sample size at each treatment and control varied from 31 to 55 larvae. Both treated and control larvae were later provided with uncontaminated diet having formaldehyde and maintained individually until pupation in 2 oz plastic cups at 26±1°C and 60±10% rh. The pupae were maintained in groups of 15—20 in sterilized rearing bottle (500 ml). Observations were recorded daily regarding larval and pupal mortality due to polyhedrosis and other causes, and adult emergence. All cases of larval or pupal mortality were confirmed by microscopic examination (× 240) of tissues for the presence or absence of polyhedra. The results were analysed statistically by "z" test after SNEDECOR (1961). The percentages of larval mortality were adjusted after ABBOTT (1925) using mortality for treated and untreated controls. The parameters like incubation period (IP) and mean time for total larval death (MTLD) (in day) following different treatments were calculated.

RESULTS AND DISCUSSION

Results are presented in Table 1. Although the results of larval mortality (24—55.7%) due to polyhedrosis produced by the virus in soil sample subjected to different treatments up to 28 or 64 weeks were erratic and varied somewhat, their comparison showed no significant difference except that between 64-week-soils in pots 1 and 3. This suggests that *M. separata* NPV can survive without much alteration in its original infectivity for a long period (for at least 28 or 64 weeks) in the present soil sample. The percentages of larval mortality and mortality rates were not as high as expected. A possible explanation is that the polyhedra in soil-water mixtures on standing for an hour (as has been done before recovering the supernatant from beakers) must have settled along with soil particles and hence the supernatant fed to the larvae contained presumably less number of polyhedra. Nevertheless, it was known that the virus-treated soil used in bioassay tests contained approximately 1.5×10^7 PIB/g.

Although the percentages of larval mortality due to the soil-laden virus were consistent in most treatments, their variation in other treatments could be accounted to the experimental error that might have occurred while recovering the supernatant from beakers. The error was evident from varied depths of sediments in stored samples of supernatant fed to the larvae after thorough shaking. Obviously, although not significant, the percentages

TABLE 1 Stability of the nonpurified *M. separata* NPV in black soil maintained in different conditions.

Duration of the virus retained in soil (week)	No. of larvae	Per cent mortality[a] due to NPV			Incubation period (day)[b]	MTLD (day)	Per cent pupation	Per cent adult emergence
		Larval	Pupal	Total				
1	2	3	4	5	6	7	8	9
Pot 1. Moistened soil kept in darkness								
1	55	25.5	2.7	28.2	8.5	10.0	67.3	97.3
4	43	33.6	0.1	33.7	8.5	11.0	60.4	88.5
12	40	36.4	22.3	58.7	8.0	11.0	57.5	60.9
28	51	25.8	0.0	25.8	9.0	11.4	70.6	100.0
64	41	33.0[c]	11.5	44.5	10.0	12.0	63.4	88.7
Pot 2. Dry soil kept in darkness[e]								
12	42	29.7	3.9	33.6	10.0	14.5	61.9	84.6
28	31	44.8	2.5	47.3	9.0	12.0	51.6	93.7
64	32	55.7[d]	23.1	78.8	10.0	13.5	40.6	84.6
Pot 3. Moistened soil kept outdoors[f]								
1	40	36.4	0.0	36.4	9.0	12.0	60.0	91.7
4	40	26.4	0.0	26.4	8.0	11.0	70.0	100.0
12	40	26.4	3.4	29.8	8.0	9.5	70.0	92.9
28	40	24.0	0.0	24.0	8.0	12.0	62.4	100.0
Pot 4. Dry soil kept outdoors[g]								
12	46	20.3	4.8	25.1	9.0	12.0	76.2	65.7
28	55	34.6	2.9	37.5	9.0	12.5	61.8	97.0

a. Mortality data were adjusted after ABBOTT (1925) for mortality of soil—extract (3.6%) and distilled water treated (2.1%) controls due to polyhedrosis. Mortality due to other causes for both the treated larvae and pupae and the same due to NPV and other causes for both the control larvae and pupae rarely exceed 8.3%.

b. For larval mortality.

e. Periods 1 and 4 weeks; f. 64 weeks; and g. 1, 4 and 64 weeks were not tested. Comparison of larval mortality (z test) : c—d : $P < 0.05$ S.

of larval mortality varied according to the amount of virus-contaminated soil that had been passed on to the supernatant. Mortality due to other causes for the treated and control larvae rarely exceeded 4.8%. The comparison of IP (8.5—10 days) or MTLD (10—14.5 days) between the treatments, and treatments and control showed no significant difference.

Pupal mortality due to polyhedrosis (delayed effect of NPV) was very low (maximum of 11.5%) in all but treatments of 12-week-soil in pot 1 (22.3% and 64-week-soil in pot 3 (23.1%). This indicates that a very slight amount of virus had been contracted from larvae to pupae. In other words, much of the NPV effect was expressed in larvae. Mortality due to other causes rarely exceeded 8.3%.

The total mortality (larval and pupal combined) figures (24—78%) are generally less significant, although such values would highly justify the importance of microbial control wherein the disease may be expressed not only in larval stage but also in subsequent stages of insect development.

NPVs and GVs of several insect species have been shown to remain stable for long periods in soil maintained outdoors or in laboratory (see TANADA, 1971: see JAQUES, 1975, 1977; HUKUHARA *et al.* 1978; THOMPSON & SCOTT, 1979). Our findings revealing the long-term stability of *M. separata* NPV. besides confirming these results, indicated a remarkable resistance of the polyhedral and/or viral protein coat to decomposition in the present soil sample despite the latter was maintained dry, moist, outdoors, or in darkness.

The foregoing account on *M. separata* NPV obviously reveal that the virus can remain stable with little or no loss of activity for long periods in soil despite the latter is exposed to various physical factors of the environment. Further, it is suggested that in the field environment the soil-borne virus of the armyworm may contaminate the lower leaves of the host plant by splashing the soil due to rain. This fact is particularly obvious when the crop is young.

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THE PROBABLE NEUROSECRETORY CONTROL OF VITELLOGENESIS IN THE MILLIPEDE, *JONESPELTIS SPLENDIDUS* VERHOEFF (DIPLOPODA : MYRIAPODA)[1]

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(Received 28 June 1980)

The effect of bilateral extirpation of the neurohaemal organs, connective bodies, on oocyte development and vitellogenesis was studied in *Jonespeltis splendidus* (Diplopoda : Myriapoda). Bilateral ablation of connective bodies from newly moulted animals resulted in the failure of previtellogenic oocytes to develop and they underwent degeneration. In the well-tanned animals which had previtellogenic and vitellogenic oocytes, extirpation of connective bodies blocked further development of oocytes and they also degenerated. Removal of connective bodies apparently affected the synthetic activities of the germinal vesicle and there was also an inhibition of the appearance of lipid, carbohydrate and protein yolk in the cytoplasm of developing oocytes. The cytological changes that occurred during degeneration in previtellogenic and vitellogenic oocytes have also been described

(*Key words:* neurosecretory control, vitellogenesis, neurohaemal organs, connective bodies, Diplopoda, *Jonespeltis splendidus*, oocyte development)

INTRODUCTION

Although considerable evidence for the hormonal factors controlling oocyte development and vitellogenesis is available in insects and crustaceans, information concerning this aspect of myriapod endocrinology is scanty. There is some evidence in the chilopod *Lithobius forficatus* suggesting that neurosecretory cells of pars intercerebralis region of the brain produce a gonadotropic factor in females (HERBAUT & JOLY, 1971; HERBAUT, 1975, 1976). No work has so far been done on similar aspects in diplopod myriapods as far as is known.

Connective bodies are a pair of neurohaemal organs of neurosecretory material elaborated by the C_1 neurosecretory cells of the brain and the neurosecretory cells of the suboesophageal ganglion (PRABHU, 1962; KRISHNAN NAIR, 1973). They are seen attached to the lateral oesophageal connectives at their origin from tritocerebrum slightly in front of the postoral commissure (PRABHU, 1962). Connective bodies were suspected to control oocyte development because these structures showed an increase in size and amount of neurosecretory material as the animals attained sexual maturity (PRABHU, 1962). The present investigation has been carried out to find out the effect of extirpation of connective bodies on oocye development in the diplopod *Jonespeltis splendidus*.

Earlier histological and histochemical studies showed that oocyte development in *Jonespeltis* consisted of four stages: previtellogenesis - A, previtellogenesis - B, vitellogenesis - A and vitellogeneesis - B (KRISHNAN NAIR, 1980). The previtello-

[1] Dedicated to the late Professor K. K. Nayar.

[2] Part of the thesis submitted for Ph. D. to the University of Kerala.

genic oocytes were characterised by an increase in the synthetic activities of germinal vesicle. During vitellogenic stages there was a rapid increase in the size of oocyte, and carbohydrate, lipid and protein yolk were laid successively in the cytoplasm of developing oocytes.

MATERIALS AND METHODS

Newly moulted and well-tanned female specimens of *Jonespeltis splendidus* VERHOEFF (Myriapoda : Diplopoda) measuring 30 to 40 mm in length freshly collected locally were used in the present study. Newly moulted animals were easily distinguishable because of their pale white and fragile body wall. The well tanned animals on the other hand had a hard, dark, pigmented and strong exoskeleton. The connective bodies were exposed and extirpated by means of extra fine (No. 5) tweezers. The wound was closed by means of a filter paper closely fitting into it. Small amounts of a mixture of penicillin and streptomycin in the ratio 1:1 were placed on the wound before sealing to prevent infection. The whole area was sealed with molten paraffin (55 to 57°C). Sham operated animals were kept as controls. Experimental and control animals were kept in separate containers filled with soil and decaying vegetable matter.

60 newly moulted animals were used for the extirpation of connective bodies and 40 were used as controls. Four experimentals each were sacrificed on 15th and 22nd day and the remaining 20 animals were sacrificed on 30th day. An equal number of controls were also sacrificed on the same days. 55 well-tanned animals were used for the extirpation of connective bodies and 25 sham operated animals were kept as controls. Five each of the experimentals and controls were sacrificed on 15, 22 and 30 days after the operation. The rest of the experimentals and some of the controls died.

At the time of sacrifice, pieces of ovaries were dissected out, fixed in appropriate fixatives and processed for histological and histochemical procedures as described by KRISHNAN NAIR (1980). The nerve ring was dissected out at the time of sacrifice and complete removal of connective bodies was confirmed.

RESULTS

In newly moulted animals all oocytes were in previtellogenesis-A. In the present studies extirpation of connective bodies essentially blocked further develoment of oocytes in them. In well tanned animals which had previtellogenic and vitellogenic oocytes in their body cavity, removal of connective bodies stopped further development of oocytes. These oocytes and the oocytes of newly moulted animals degenerated. In both control groups the oocytes developed normally. So in both control groups the oocytes which were at the previtellogenic stage at the time of operation had undergone further development and became vitellogenic and a new generation of previtellogenic oocytes were formed. In the description that follows the cytological changes of degenerating previtellogenic oocytes of experimental animals were compared to those of the new previtellogenic oocytes of the controls in order to study the changes undergone during the degeneration process.

Effects of bilateral extirpation of connective bodies in the newly moulted animals, on the oocytes:

These experiments designed to study the role of connective bodies on initiation and continuation of oocyte development and vitellogenesis in the newly moulted animals in which oocytes were at previtellogenic stage. Examination of ovaries of the experimental animals 15 days after the operation showed that oocytes developed only till the previtellogenic stage and further growth was arrested (Fig. 1). Controls on the other hand had fully grown oocytes in their body cavity. The size of the oocytes in both experimental and control animals varied considerably; the largest oocyte of the experimental animals measured 50 μm whereas

those of the controls ranged around 200 μm in diameter.

In the oocytes of the experimental animals the germinal vesicle as a whole including the nucleolus gave a faint reaction for protein in contrast to the situation in controls where the germinal vesicle showed deeply stainable protein granules. The nucleolus of the oocytes of experimental animals having a diameter of 4 μm was peculiar in having a ring-shaped appearance (Fig. 2). It had an outer basophilic cortex and an inner less basophilic region. the medulla. Nucleolar extrusions which were characteristic of previtellogenic oocytes of controls were practically nil or absent in experimental animals.

Cytoplasm of most of the experimental oocytes was characterised by an eosinophilic perinuclear cap containing little RNA and carbohydrates but abundant proteins (Fig. 3). The rest of the cytoplasm was vacuolated. The vacuoles contained certain basophilic granules which were proteins. There were no protein granules in the oocytes of experimental animals except for a faint reaction for protein which was normally met with in tissues. There was very little RNA in the cytoplasm as opposed to abundant RNA in controls. In the oocytes of control animals yolk droplets containing protein and carbohydrates were seen.

Twentytwo to thirty days after the operation the germinal vesicle and the cytoplasm underwent more conspicuous degenerative changes in the oocytes of experimental animals. The nuclei were irregular in shape and each nucleus was surrounded by a condensed mass of uniformly and intensely stained eosinophil cytoplasm (Fig. 4). Nucleolus lost its basophilia and became acidophilic. In some of the oocytes germinal vesicle completely disappeared leaving behind a vacuole in that region. Numerous basophilic particles could also be seen in the degenerating cytoplasm (Fig. 5). Such degenerative changes were not visible in the oocytes of control animals (Fig. 6).

Effects of bilateral ablation of connective bodies in the well-tanned animals, on the oocytes:

Normally in the breeding season the ovaries of well-tanned animals contained oocytes at different stages of vitellogenesis. Removal of connective bodies not only disturbed further growth of the oocytes but they also underwent degeneration. Oocytes started degeneration either in the previtellogenic or in the vitellogenic stage.

Degeneration of previtellogenic oocytes consisted of a series of processes. Fifteen days after the extirpation of connective bodies the cytoplasm of previtellogenic oocytes showed certain eosinophil inclusions distributed among numerous basophilic particles (Fig. 7). Thus the cytopalsm became highly acidophilic unlike the case in control animals where the cytoplasm of previtellogenic oocytes was intensely basophilic. The nucleolus in the majority of cases was ring shaped (Fig. 7). In the previtellogenic oocytes 22 days after the operation the germinal vesicle became surrounded by an eosinophil cytoplasm (Figs. 8, 9 & 10). Nucleolar emissions of the oocyte of experimental animals were very much reduced in number in contrast to those of the controls where the nucleolus was homogeneously basophilic and showed numerous nucleolar particles. Thirty days after the removal of connective bodies the germinal vesicle of the oocytes disappeared and the cytoplasm also degenerated.

In the vitellogenic oocytes several degenerative changes could be noticed on 15, 22 and 30 days after the operation. The largest oocyte of the experimental animals

measured 200 μm whereas the largest oocyte of control animals measured 400 μm in diameter. The yolk granules measuring 20 μm showed considerable difference to that of the control where they measured 35 μm in size. The protein yolk lost its granular shape and broke down into small spherules. The chorion became folded and at advanced stages of degeneration chorion disappeared (Fig. 11), and masses of degenerating yolk could be distinguished among degenerating previtellogenic oocytes (Fig. 12).

DISCUSSION

The present studies revealed that bilateral removal of connective bodies completely inhibited the development of oocytes in newly moulted as well as in well-tanned animals. Removal of connective bodies from newly moulted animals essentially blocked further development of oocytes and the oocytes underwent degeneration. In the well-tanned animals with oocytes at different developmental stages removal of connective bodies arrested further growth of oocytes and these as well as other oocytes in which vitellogenesis has already taken place, underwent degeneration. It thus seemed that connective bodies were necessary not only for the development of oocytes but also for the maintenance of vitellogenic oocytes.

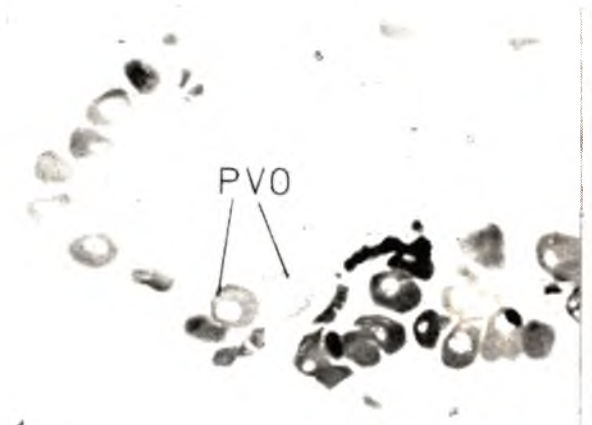
The activity of the germinal vesicle was very much inhibited as a result of the operation. The transformation of compact and dense nucleoli of the oocytes of *Jonespeltis* into ring shaped less basophilic condition of the oocytes of the experimentals could represent a cessation of RNA synthesis by the nucleoli (JOURNEY & GOLDSTEIN, 1961; PANTELEAKIS & MAIEYKO, 1966; SMETANA & POTMESIL, 1968; TERAO *et al.*, 1971; BURNS & SOLOFF, 1972). This diminution of the activity of the nucleolus was further reflected in the decrease of nucleolar extrusions which again partly explained the disappearance of RNA and protein in the cytoplasm. Extirpation of connective bodies affected the disappearance of yolk in the cytoplasm of the oocytes. There was an inhibition of the formation of carbohydrate, lipid and protein yolk.

Vacuoles described in the degenerating oocytes had the appearance of digestive vacuoles described in the resorbing oocytes of insects (LUSIS, 1963; BITSCH, 1968). Hydrolytic enzymes present in these vacuoles appear to be responsible for the lysis of the oocytes (LUSIS, 1968).

The results of the present experiments suggest that connective bodies control yolk deposition and its maintenance in *Jonespeltis*. Connective bodies were considered to be the neurohaemal organs of two sources of neurosecretion, one from the brain and the other from the suboesophageal ganglion (KRISHNAN NAIR, 1973). It could therefore be concluded that the gonadotropic activity that might be attributed to the

EXPLANATION OF FIGURES

Figs: 1. Ovary of newly moulted animal 15 days after extirpation of connective bodies showing oocytes in the previtellogenic stage, × 100. 2. A group of previtellogenic oocytes of newly moulted animal 15 days after the extirpation of connective bodies showing the ring shaped nucleolus, × 400. 3. Oocyte of newly moulted animal 15 days after the removal of connective bodies showing the perinuclear eosinophilic cap, × 400. 4. A group of previtellogenic oocytes of newly moulted animal 22 days after the removal of connective bodies showing the perinuclear eosinophilic cap, × 400. 5. Ovary showing the degenerating nuclei of previtellogenic oocytes, 22 days after the removal of connective bodies from newly moulted animal, × 100. 6. Ovary of the newly moulted control animal 30 days after the operation showing previtellogenic and vitellogenic oocytes, × 100. Figs. 1 to 6. Carnoy's fluid, Heidenhain's haematoxylin-eosin. PVO-Previtellogenic oocytes, GV—Germinal vesicle, PNCY—Perinuclear eosinophilic cap, DCY—Degenerating cytoplasm. VO—Vitellogenic oocyte, RN—Ring shaped nucleolus, N—Nucleolus, CY—Cytoplasm.



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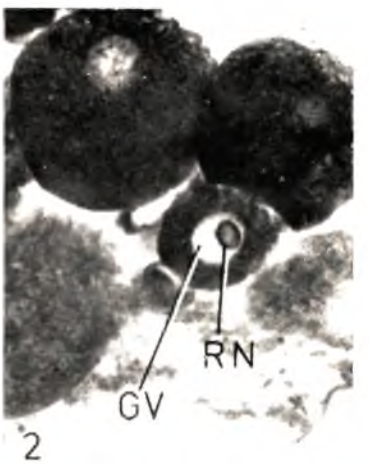

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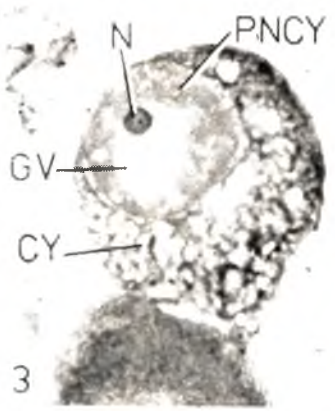

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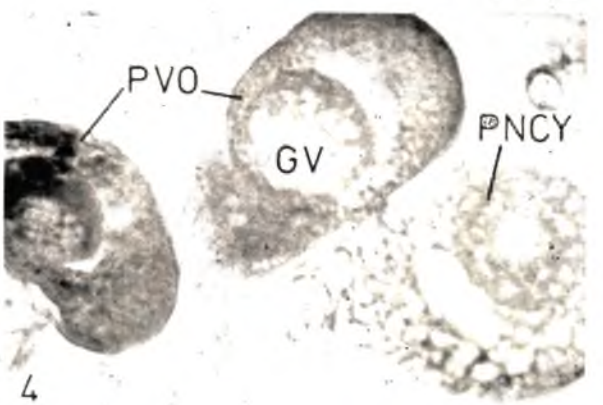

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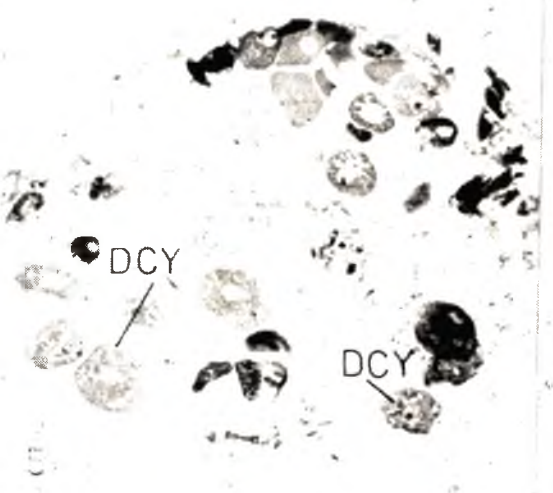

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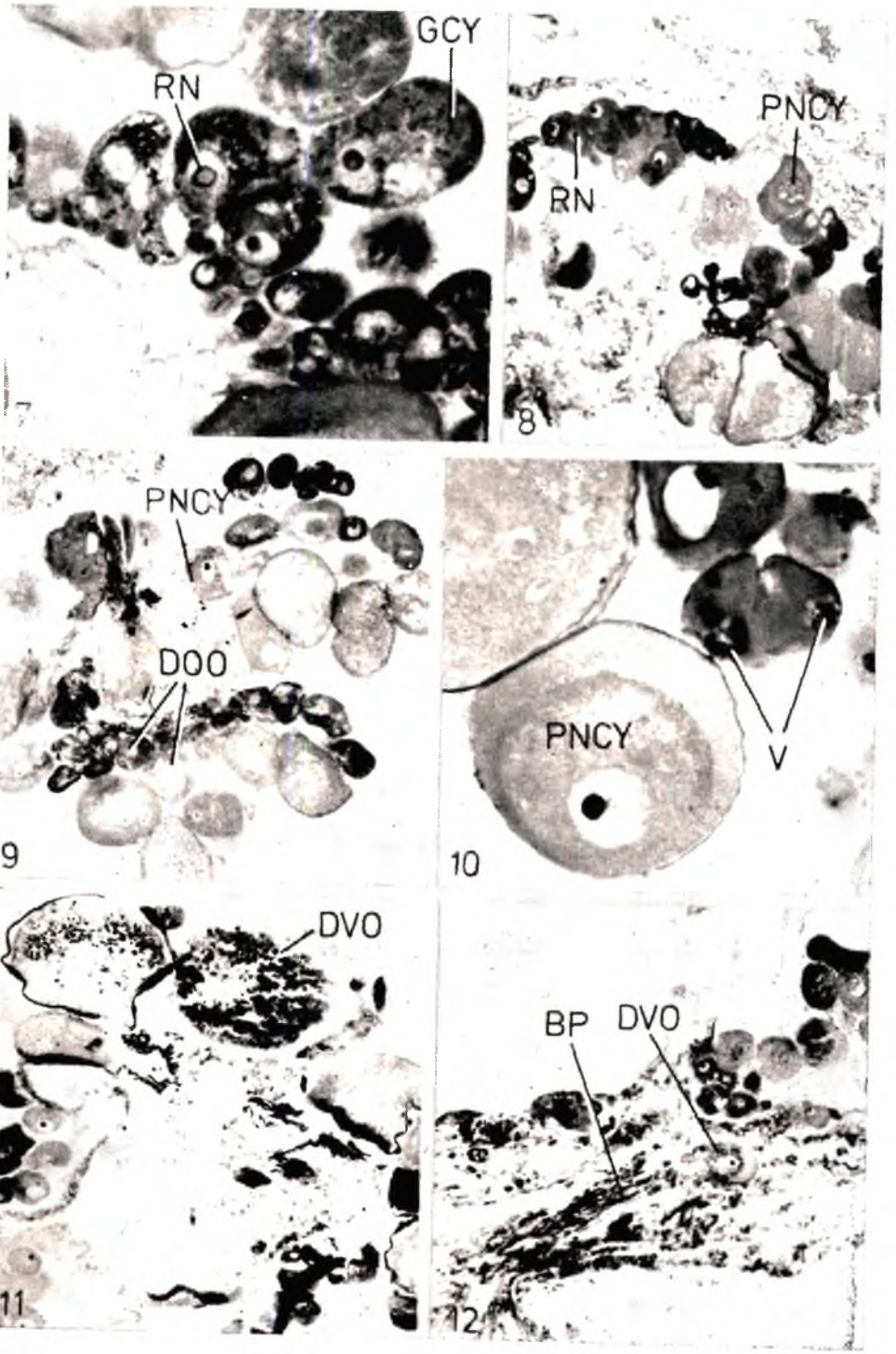
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connective bodies was either due to a neurosecretory factor from the brain or from suboesophgeal ganglion or both. In chilopods similar neurosecretory factors from the brain has been shown to control vitellogenesis (HERBAUT & JOLY, 1971; HERBAUT, 1975, 1976) and spermatogenesis (DESCAMPS, 1974).

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Figs: 7. Ovary of well-tanned animal 15 days after the removal of connective bodies, showing the granular nature of the cytoplasm of previtellogenic oocytes, × 400. 8. Ovary of well-tanned animal 22 days after the removal of connective bodies, × 100. 9. Ovary of well-tanned animal 22 days after the removal of connective bodies showing the degenerating oocytes, × 100 10. Ovary of well-tanned animal 22 days after the removal of connective bodies showing the eosinophil perinuclear cytoplasm, × 400. 11. Ovary of a well-tanned animal 15 days after the removal of connective bodies showing degenerating vitellogenic oocytes. Note disappearance of the chorion, × 100. 12. Ovary of a well-tanned animal 30 days after the removal of connective bodies showing degenerating oocytes, × 100. Figs. 7 to 12, Carnoy's fluid, Heidenhain's haematoxylin- eosin. GCY—Granular cytoplasm, RN—Ring shaped nucleolus, PNCY—Perinuclear eosinophilic cytoplasm, DOO—Degenerating oocytes, V—Vacuoles, DVO—Degenerating vitellogenic oocytes, BP—Basophilic particles.

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THE EFFECT OF DIFLUBENZURON ON THE CASTOR SEMILOOPER *ACHOEA JANATA* LINN. (LEPIDOPTERA : NOCTUIDAE)

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Diflubenzuron disrupted the growth and metamorphosis in larvae of *Achoea janata* Linn. Even the lowest concentration tested (0.05 g litre^{-1}) inhibited moulting and caused mortality to the extent of 96.0 per cent. Concentrations above 0.05 g litre^{-1} resulted in 100 per cent mortality. Due to treatment with the compound, various morphological deformities were observed in the pupae. Histological sections of treated insects revealed irregular deposition of cuticle

(*Key words:* diflubenzuron effect, castor semilooper, *Achoea janata*, growth and metamorphosis disruption, moulting, morphological deformity, mortality, irregular cuticle deposition)

INTRODUCTION

Diflubenzuron reduces the rate of production of chitin during cuticle deposition by competitive inhibition of the final enzyme, chitin synthetase (DUEL *et al.*, 1978). This compound has been found to interfere with the growth and development of a number of insect pests of agricultural importance (POST & VINCENT, 1973: SUNDARAMOORTHY & SANTHANAKRISHNAN, 1979: MOKROUSOVA, 1977). This paper deals with the effect of diflubenzuron on the castor semilooper, *Achoea janata* Linn. a serious seasonal pest on castor, rose and pomegranate.

MATERIALS AND METHODS

Larvae of *A. janata* obtained from eggs of laboratory reared moths were raised on castor leaves. Diflubenzuron, 1 - (4 - chlorophenyl) - 3 - (2,6 - diflurobenzoyl) urea in the form of *Dimilin* (Philips-Duphar B. V., Holland) was tested on the final instar larvae. Four concentrations of diflubenzuron (0.05 g litre^{-1}, 0.1 g litre^{-1}, 0.5 g litre^{-1}, and 1.0 g litre^{-1}) were prepared from dimilin and clean castor leaves were dipped in these solutions for 15 seconds and air dried. Early final instar larvae of uniform size were starved for 6 hr and allowed to feed on dimilin-treated leaves for 24 hr. Fresh untreated leaves were supplied after 24 hr and the development of the insects was observed. In each concentration, twenty five larvae were inoculated with three replications.

Portions of treated insects in prepupal stage were fixed in alcoholic Bouin's fixative and after removing excess picric acid with 70% ethyl alcohol, were dehydrated end embedded in paraffin wax according to standard procedures. Sections cut at 4—6 μm were stained in Heidenhain-iron hematoxylin with eosin - Y as the counter stain.

RESULTS AND DISCUSSION

Feeding of the last instar larvae of *A. janata* on dimilin treated castor leaves inhibited the normal growth and metamorphosis of the insect, resulting in various morphological deformities in the

pupae (Fig. 1). Though the deformed insects survived for 2-3 days, they did not survive to develop into adults. Such deformities have been reported in diflubenzuron-treated larvae of *Spodoptera litura* (SUNDARAMOORTHY, 1977) and *Nephantis serinopa* (SUNDARAMOORTHI & SANTHANAKRISHNAN, 1979). Certain affected specimens of *A. janata* were distinctly larval-pupal mosaics (Fig. 1, C-E). Further, in certain parts of the deformed pupae especially on the ventral side, deposition of cuticle was incomplete. Histological sections clearly showed the absence of exocuticle in such areas. Under the influence of diflubenzuron, stable layer of cuticle is not deposited as reported in *Pieris brassicae* by MULDER & GIJSWIJT (1973). However, the suppression of cuticle formation is not uniform throughout the insect and only from some regions of the insects is it possible to obtain affected cuticle as reported by KER (1978). This type of irregular disruption of cuticle deposition could be one of the possible reasons for the formation of mosaic forms of diflubenzuron-treated *A. janata.*

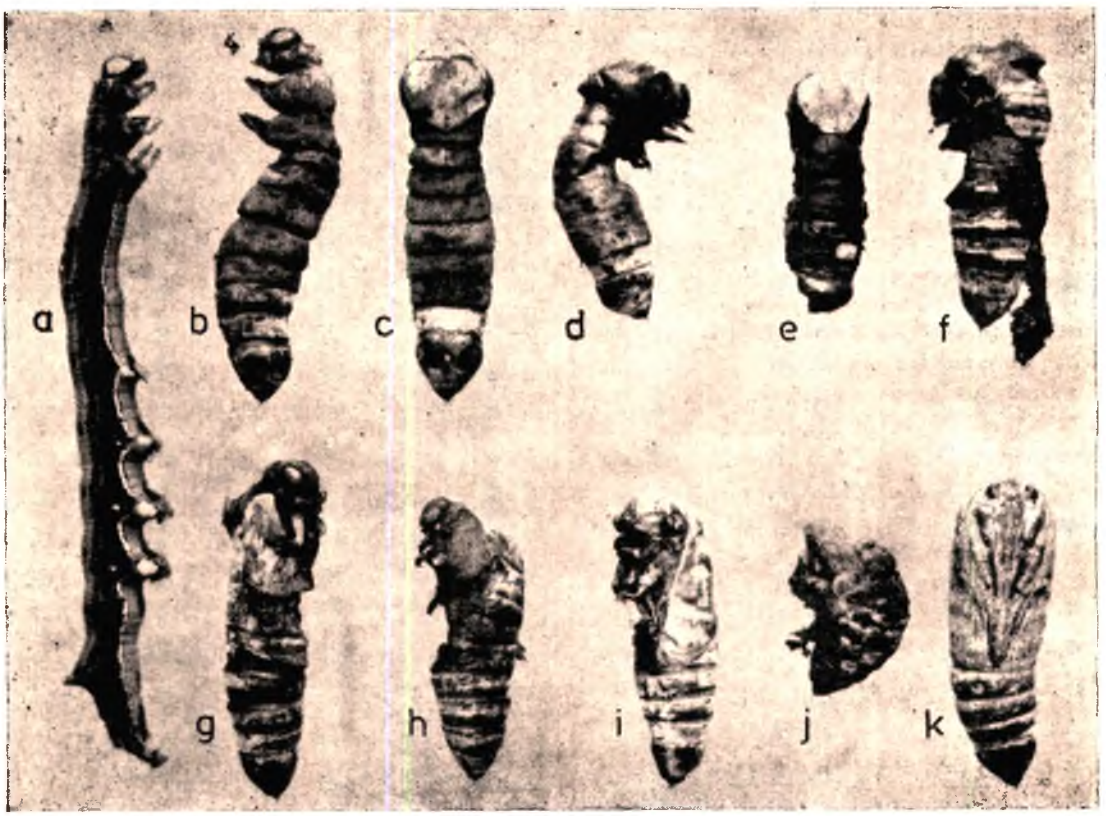

Figure 1. Morphogenetic effects of Diflubenzuron on pupae of *Achoea janata.* a) Normal larva b) Larviform pupa with anterior portion completely larval c-e) Larval-pupal mosaics f) Forwardly bent pupa with larval head and two pairs of legs. g) Elongate pupa with short wing pads on one side only; sclerotised cuticle absent ventrally in the mid portion h) Twisted larval-pupal intermediate. i) An almost normal pupa with larval head and legs. j) Curved pupa k) Normal pupa

TABLE 1 Toxicity of different concentrations of diflubenzuron to final instar larvae of *Achoea janata*.

Mortality as	Mean frequency (%)			
	0.05 g litre^{-1}	0.10 g litre^{-1}	0.5 g litre^{-1}	1.0 g litre^{-1}
Prepupa	32.0	40.0	44.0	40.0
Larval-pupal intermediate in larval skin	44.0	48.0	16.0	36.0
Larval-pupal intermediate	20.0	12.0	40.0	24.0
Total mortality	96.0	100.0	100.0	100.0

In the abnormal forms, the posterior portion was invariably pupal and only in the anterior region larval features were retained. The cuticle in these areas was highly fragile due to reduction in the tensile strength or due to the lack of well sclerotisation. Treated insects died either as prepupae, larval-pupal intermediate forms enclosed in larval skin or as larval-pupal intermediate forms (Table 1). The concentrations tested did not seem to influence the stage at which mortality occurred. In a few prepupae, below the larval skin, patches of dark brown and well sclerotised pupal integument were observed. This is most likely due to inhibition of moulting of the larvae on one hand and the irregular deposition of cuticle on the other as diflubenzuron inhibits ecdysone metabolism (YU & TERRIERE, 1977) and ecdysterone controls chitin deposition (KIMURA, 1973).

The lowest concentration tested (0.05g litre^{-1}) could bring about a very high mortality rate of 96.0 per cent (Table 1). Concentrations above 0.05 g litre^{-1}) caused 100 per cent mortality. In the case of *Nephantis serinopa*, SUNDARAMOORTHY & SANTHANAKRISHNAN (1979) found that the maximum concentration tested (4.0 g litre^{-1}) could produce only 75 per cent mortality. This shows that diflubenzuron is more toxic to *A. janata* and even at low concentrations the compound could be used for the suppression of these insects.

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A PHYSIOLOGICAL SALINE FOR *TROGODERMA GRANARIUM* EVERTS (COLEOPTERA : DERMESTIDAE)

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A physiological solution with mM concentration of NaCl—146, KCl—7.8, $CaCl_2$—3 and $MgSO_4$—1.3 in 10 mM phosphate buffer (pH 7.2) was found suitable for semi-*in vitro* studies in a stored grain pest, *Trogoderma granarium* Everts. Cytophysiological analyses such as diameter of plasmatocytes, plasmatocyte nuclei and ^{3}H-lysine incorporation into total extractable proteins of larvae have been utilized for comparison of: suitability of the test medium with other media of different ionic patterns, osmolarity and pH.

(*Key words:* physiological saline, semi-*in vitro* studies, *Trogoderma granarium*, cytophysiological analyses, plasmatocyte, ^{3}H-lysine incorporation)

INTRODUCTION

The last larval instar of *Trogoderma granarium* EVERTS shows facultative diapause (BURGES, 1959). Though a few *in vivo* studies have been reported on *Trogoderma* larvae during diapause (GUPTA 1976; GUPTA & AGARWAL, 1976), its biochemical aspects have not been fully studied (GUPTA & AGARWAL, 1976). Small size of *Trogoderma* larvae, their low haemolymph volume and their highly diffuse fat bodies are some of the main reasons for this lacuna. In many insects *in vitro* systems have been utilized for studying biochemical changes during metamorphosis and diapause (VAUGHN, 1971; QUIOT, 1971; FLORKIN & JEUNIAUX, 1974). However, in *Trogoderma* such *in vitro* studies during diapause have not been successful for lack of a suitable incubation medium. In our laboratory none of the commonly used media was found suitable for *in vitro* studies. We, therefore, worked out a medium which can be profitably utilized for semi-*in vitro* studies in *Trogoderma* and the same is described here.

MATERIALS AND METHODS

Trogoderma larvae used for this work were drawn from cultures maintained on broken wheat according to the procedure described earlier by GUPTA & AGARWAL (1976). Diapause larvae exposed to 45 hr of diapause terminating conditions were used in order to study the effects of various test media on their cytophysiology. Such larvae showed a high rate of amino acid incorporation (GUPTA & AGARWAL, 1976) and increased number of plasmatocytes, a well defined class of haemocytes. These cells were identified by their clearly demarcated nuclei and cytoplasmic area (JONES, 1977). The media preparations are given in Tables 1 and 2.

Incorporation of ^{3}H-lysine was taken as a parameter for physiological analyses. 0.1 μCi of ^{3}H-lysine (sp. activity 16 Ci/mM, BARC, Bombay) was injected per larva for *in vivo* studies using a hypodermic 30 G needle with the help of an Agla micro applicator. For semi-*in vitro* studies, five larvae were incubated in 2 ml of test medium containing 10 μCi ^{3}H-lysine according to the method utilized for *Tenebrio*

TABLE 1. Composition of the insect Ringer solutions tested for their suitability for semi-*in vitro* studies in *Trogoderma granarium*

insect Ringer	ion concentration in millimoles (mM)											molar indices of		Na^+/K^+	pH	reference
no. insect	Na-Cl	KCl	$Ca-Cl_2$	$Mg-Cl_2$	$Mg-SO_4$	$NaH-CO_3$	Na_2-HPO_4	NaH_2-PO_4	KH_2PO_4	glucose	osmolarity in milliosmole	Na^+	K^+	molar ratio		
1. locust bicarbonate buffer	129.8	9.9	1.4	0.92	—	4.0	—	—	5.8	—	305.96	42.42	3.23	13.1	8.0	PROSSER (1965)
2. general insect Krebs Ringer phosphate medium	122.0	3.0	1.3	—	1.2	25.0	—	—	0.4	10.0	317.10	38.47	0.94	40.6	8.3	ELLIOTT (1955)
3. *Tenebrio molitor*	128.0	5.0	3.0	—	1.3	—	10.0	10.0	—	10.0	327.6	39.07	1.52	25.6	7.2	ILLAN *et al.* (1966)
4. general insect	159.0	13.0	3.4	—	—	2.1	—	10.0	—	—	378.4	42.01	3.43	12.2	7.1	PROSSER (1965)
5. cockroach	187.0	21.0	5.1	0.63	—	—	—	—	—	—	453.19	41.26	4.63	8.9	7.0	PROSSER (1965)

TABLE 2. Modifications of the Krebs Ringer phosphate medium tested for their suitability for semi-*in vitro* studies in *Trogoderma granarium* larvae.

test medium no.	modification	ion concentration in milllimoles (mM)							osmolarity in millosmole	molar indices of		Na^+/K^+ molar ratio	pH
		NaCl	KCl	$CaCl_2$	$MgSO_4$	Na_2HPO_4	NaH_2PO_4	glucose		Na^+	K^+		
6.	changed pH	128.0	5.0	3.0	1.3	10.0	10.0	10.0	327.6	39.07	1.52	25.6	6.8
7.	lack of Ca^{2+}	128.0	5.0	--	1.3	10.0	10.0	10.0	318.6	40.17	1.56	25.6	7.2
8.	lack of phosphate buffer	128.0	5.0	3.0	1.3	—	—	10.0	287.6	44.50	1.73	25.6	7.1
9.	different Na^+/K^+ molar ratio	136.0	6.3	3.0	1.3	10.0	10.0	10.0	346.2	39.28	1.81	21.5	7.2
10.	different Na^+/K^+ molar ratio	146.0	7.8	3.0	1.3	10.0	10.0	10.0	369.0	39.56	2.11	18.71	7.2
11.	different Na^+/K^+ molar ratio	154.0	7.8	3.0	1.3	10.0	10.0	10.0	385.2	39.97	2.02	19.74	7.2

molitor (ILLAN *et al.*, 1966). The semi-*in vitro* incorporation of ^{3}H-lysine was terminated by addition of 5% trichloroacetic acid. The extent of incorporation into total extractable proteins of larvae was determined by the procedure described earlier by GUPTA & AGARWAL (1976). The *in vivo* incorporation so obtained was taken as the control value for the semi-*in vitro* incorporation of ^{3}H-lysine.

Changes in diameter of spheroidal type of plasmatocytes were taken as a parameter for cytomorphological analyses. However, other polymorphic forms of plasmatocytes were also present at the described stage of diapause but such forms could not be utilized for the analyses because of variability in their nuclei and cytoplasmic area. The diameter of these cells were recorded from formol fixed haematoxylin-eosin stained smears prepared from haemolymph diluted with test medium (experimental). Similar observations from the preparation made from undiluted haemolymph (control) were compared with those of experimental observation in order to dilute the secondary effect of fixation and staining on cytomorphology of spheroidal plasmatocytes.

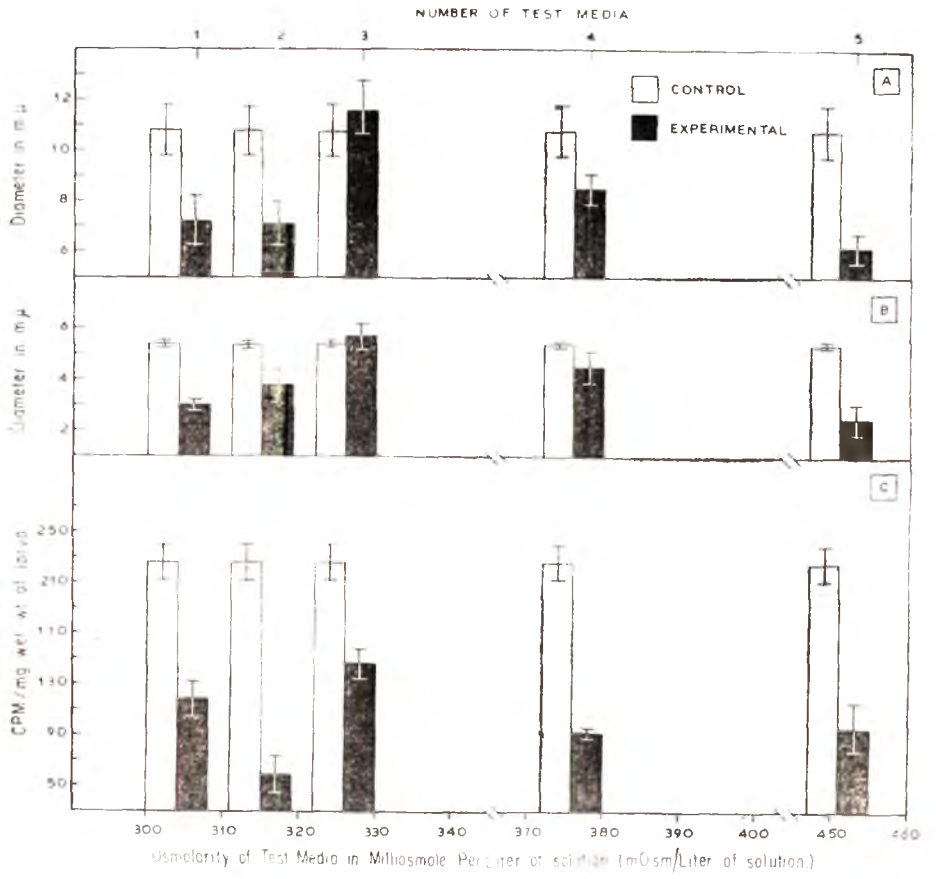

Fig. 1. Effect of total osmolarity, pH and ionic patterns on the diameter of plasmatocytes (A), diameter of plasmatocyte nuclei (B), semi-*in vitro* incorporation of ^{3}H-lysine into the total proteins of *T. granarium* larvae (C). Semi-*in vitro* observations (experimental) are compared with those from *in vivo* incubations (control). Two ml each of the medium containing 10 μCi ^{3}H-lysine was used for semi-*in vitro* studies according to ILLAN *et al.*, (1966). The incubation was for 2 hr at 37°C.

RESULTS AND DISCUSSION

Semi-*in vitro* incubations of larvae in test media Nos. 1—5 show the suitability of incubation medium for the test animal (Fig. 1). The diameter of plasmatocytes and their nuclei decreased except in test medium No. 3 (Figs. 1A, B). But the incorporation of ^{3}H-lysine in all the five test media was less than the controls (Fig. 1C). However, in test medium No. 3 (Krebs Ringer phosphate medium) maximum incorporation of ^{3}H-lysine was observed. It appears from the foregoing discussion that medium No. 3 has some osmotic resemblance with that of the test animal and was thus selected for further experimentations.

Change in pH from 7.2 to 6.8 (medium No. 6) resulted in increase in diameter of plasmatocytes (Fig. 2A). But this increase in diameter of plasmatocytes was associated with decline in ^{3}H-lysine incorporation (Fig. 2C). Similar increase in size of plasmatocytes was observed when Ca^{++} ions (medium No. 7) and phosphate buffer (medium No. 8) were not present in the test medium (Fig. 2A). However, the incorporation of ^{3}H-lysine in these conditions was less than those of their corresponding controls (Fig. 2C). But the

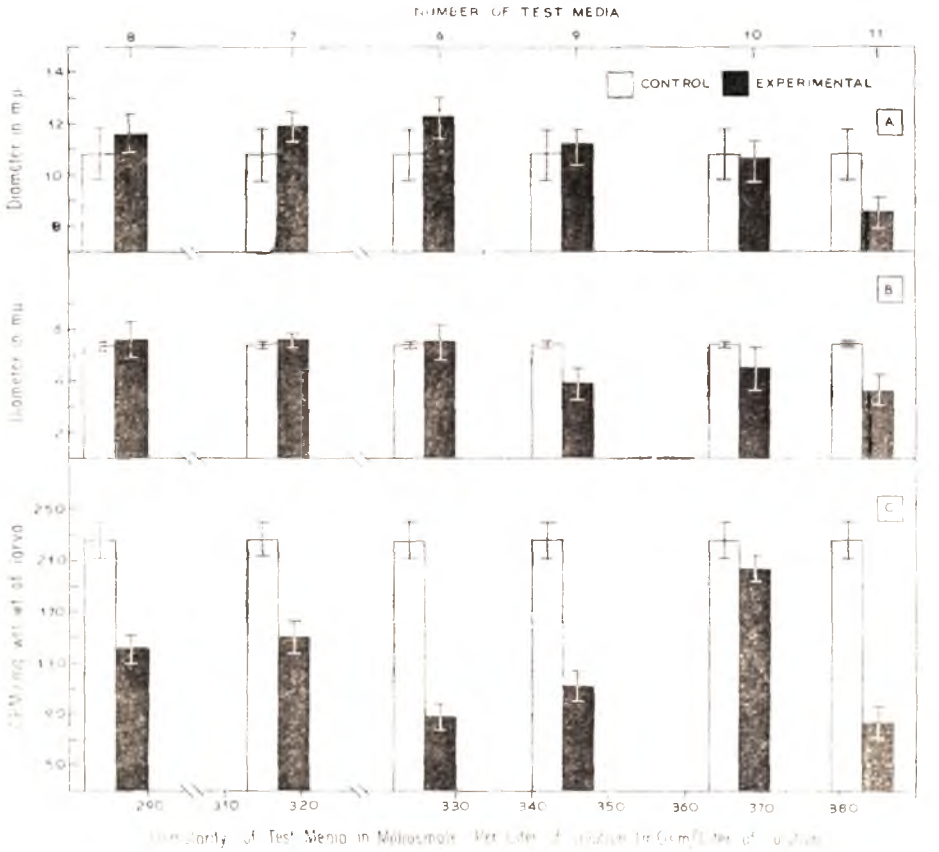

Fig. 2. Effect of cationic and pH modifications in Krebs Ringer phosphate medium on diameter of plasmatocytes (A), diameter of plasmatocyte nuclei (B), semi-*in vitro* incorporation of ^{3}H-lysine into the total proteins of *T. granarium* larvae (C). Conditions for incubations etc. were the same as in Fig. 1.

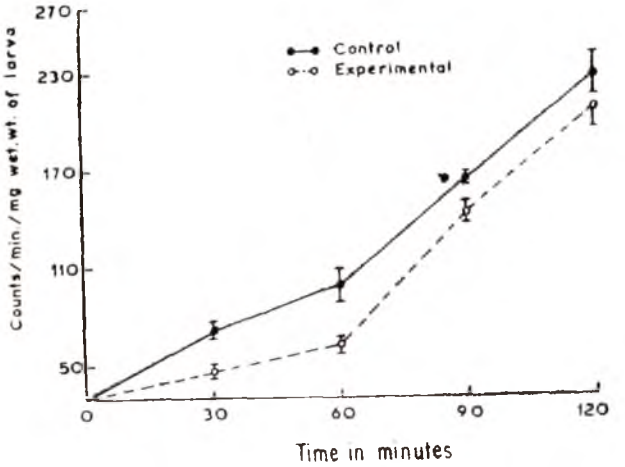

Fig. 3. Comparison of semi-*in vitro* (experimental medium no. 10) and *invivo* (control) ^{3}H-lysine incorporation patterns into total extractable proteins of *T. granarium* larvae. Conditions for incubations etc. were the same as in Fig. 1 except time.

rate of such incorporation of ^{3}H-lysine was much higher than that obtained in changed pH conditions. ILLAN *et al.*, (1966) in *Tenebrio molitor* also recorded decline in macromolecular syntheses in absence of phosphate buffer from the similar semi-*in vitro* incubations.

It is interesting to note the condition of plasmatocytes and rate of ^{3}H-lysine incorporation with the changes in Na^{+}/K^{+} molar ratio of the incubation media (Test medium No. 9, 10 and 11: Fig. 2). There was a substantial increase in the incorporation of ^{3}H-lysine accompanied with a slight decline in diameter of plasmatocyte when the Na^{+}/K^{+} molar ratio was changed from 21.5 to 18.7. However, at 19.7 Na^{+}/K^{+} molar ratio, remarkable decrease in the incorporation of ^{3}H-lysine and diameter of plasmatocytes was observed (Fig. 2). These observations tend to show that sensitivity of the medium for semi-*in vitro* studies is dependent on its Na^{+} K^{+} molar ratio. Similar results have been recorded in several other insects (FLORKIN & JEUNIAUX, 1974). The pattern of incorporation of ^{3}H-lysine in relation to different time intervals of semi-*in vitro* incubation was also compared with that obtained in *in vivo* conditions (Fig. 3). The rate of incorporation in semi-*in vitro* studies was found to be slightly lower than the controls at all times of incubations (Fig. 3). However, at 2 hr of incubation the comparison of such incorporation was found to be statistically insignificant ($p > 0.50$). PRICE (1967) reported a similar decreased incorporation of radioactive precursors into various macromolecules in *in vitro* studies with the fat bodies of *Calliphora erythrocephala*. Many factors such as injury, natural ionic bindings in the haemolymph and high anionic molar indices in the test medium have been implicated for such a decline in the incorporation of radioactive precursors into macromolecules (VAUGHN, 1971; QUIOT, 1971).

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THE POISON GLAND OF *POLISTES HEBRAEUS* (POLISTINAE, VESPIDAE): MORPHOLOGY AND CYTOCHEMISTRY

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The cellular components and cytochemistry of the poison gland of the wasp, *Polistes hebraeus* were studied. Histological studies revealed the presence of a convoluted poison gland enclosed within the lumen of the storage reservoir in addition to the free poison gland filaments that leave the reservoir at its anterior end. The cells of the convoluted gland possess intracellular reservoirs for the accumulation of secretion. The secretion is a complex mixture of proteins-especially those with bound NH_2 groups and phosphoiipids.

(Key words: morphology, cytochemistry, poison gland, *Polistes hebraeus)*

INTRODUCTION

In many hymenopterans the accessory reproductive glands in the female have become modified to serve a defensive function. HERMANN (1968 a, b; 1969 a, b) has published a series of papers on the hymenopteran poison apparatus. Besides being restricted to the ant families these have mostly emphasized on the sclerites and musculature. Detailed cytological and cytochemical studies on the gland responsible for poison secretion are lacking. CALLAHAN *et al.* (1959) have studied the poison apparatus of the imported fire ant *Solenopsis saevissima*. Quite a few reports are available regarding the poison apparatus in honey bees (ECKERT, 1950; OWEN & BRIDGES, 1976; KANWAR & BRAR, 1976a, b) but very little is published about the poison apparatus in wasps. A few workers (RATCLIFFE & KING 1969; KANWAR & SETHI, 1971; KANWAR & KANWAR, 1975; KANWAR & BRAR, 1976 a, b) have recently reported the cytochemistry of the poison gland in some hymenopterans but the vast majority still remain unexplored. The present paper deals with the cellular make-up of the poison gland complex and the cytochemistry of its inclusions in the yellow wasp, *Polistes hebraeus*.

MATERIAL AND METHODS

The wasps—*Polistes hebraeus*—were collected from the Panjab University campus at Chandigarh, usually from the nests of this social hymenopteran. All dissections were performed in physiological saline (0.7% NaCl, to every 100 ml of which 1 ml of 0.2% $CaCl_2$ was added). Immediately after dissection the poison glands were transferred to a variety of fixatives (Zenker, Helly, Bouin, Carnoy, neutral formalin, formaldehyde calcium and weak Bouin) suited to the cytological and cytochemical studies. Paraffin sections were cut at 6 μm while gelatin sections were cut at 10 μm.

RESULTS

The defense apparatus in *Polistes hebraeus* comprises (i) paired, free, tubular, acid glands also referred to as the free poison gland filaments; (ii) a single median reservoir; (iii) a convoluted poison gland which is enclosed within the reservoir; and (iv) an ejaculatory duct which

leads from the base of the reservoir to the sting (Fig. 1). Present only in the female, the apparatus occupies the last three abdominal segments.

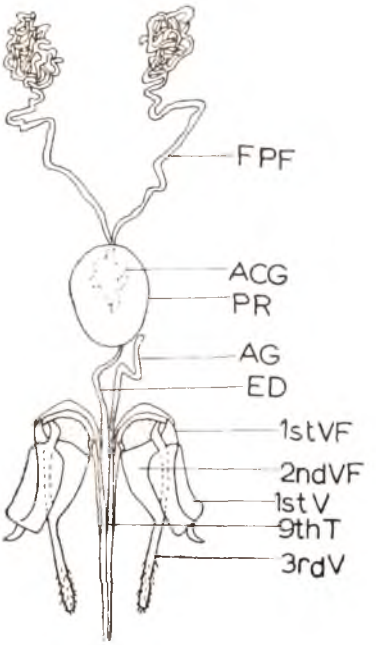

FIG. 1

Semidiagramatic representation of the poison apparatus of *Polistes hebraeus*. ACG- area of convoluted gland; AG- alkaline gland; ED- ejaculatory duct; FPF-free poison gland filament; PR- poison reservoir; 9thT- 9th tergum; 1st V- 1st valvula; 3rdV- 3rd valvula; 1st VF- 1st valvifer; 2ndVF- 2nd valvifer.

The tubular acid glands are two, long, filamentous structures that lie coiled and entangled in a mass of tracheoles and fat bodies. These arise from the anterior tip of the reservoir but curving outwards and downwards, they come to lie on either side of the reservoir near its base. They have a uniform diameter almost throughout but become narrow just before entering the reservoir.

The secretory cells of the free poison gland filaments are wedge-shaped structures arranged in a single layer around the cuticularised lumen of the central collecting duct (Figs. 2 & 3). Each of these cells exhibits almost homogeneously dense cytoplasm in which can be differentiated some granules of different sizes, a few hyaline vesicles, rarely some vacuoles and a large basal nucleus with a prominent nucleolus and granular chromatin. Branches of a fine cuticular ductule permeate the apical portions of the secretory cells to drain the secretory products into the lumen of the central duct. In addition to wedge-shaped secretory cells, there are present a few small squamous epithelial cells—the chitogenous cells—closely apposed to the lumen. Less frequently these may be interposed between the apices of the large wedge-shaped cells and are then referred to as the interstitial cells.

The reservoir is a muscular sac consisting of striated muscle fibres arranged in four bundles. It is lined on the inside by a layer of squamous epithelium resting on a well developed cuticle. The reservoir encloses in its lumen the convoluted poison gland (Figs. 4 & 5).

The free tubular gland and the convoluted gland are, apparently, parts of the same structure but they have different cellular components. The cells constituting the convoluted poison gland are small and polygonal. A small bulb-like structure can be distinguished in the cytoplasm of these cells (Fig. 5). This is a reservoir serving to store the secretion within the cell. A cuticle lined convoluted duct traverses the convoluted gland and portions of it can be seen in a transverse section to contain globules of secretion.

The ejaculatory duct leaves the reservoir at its posterior end. It is lined by a thick layer of cuticle which is thrown into small teeth-like projections.

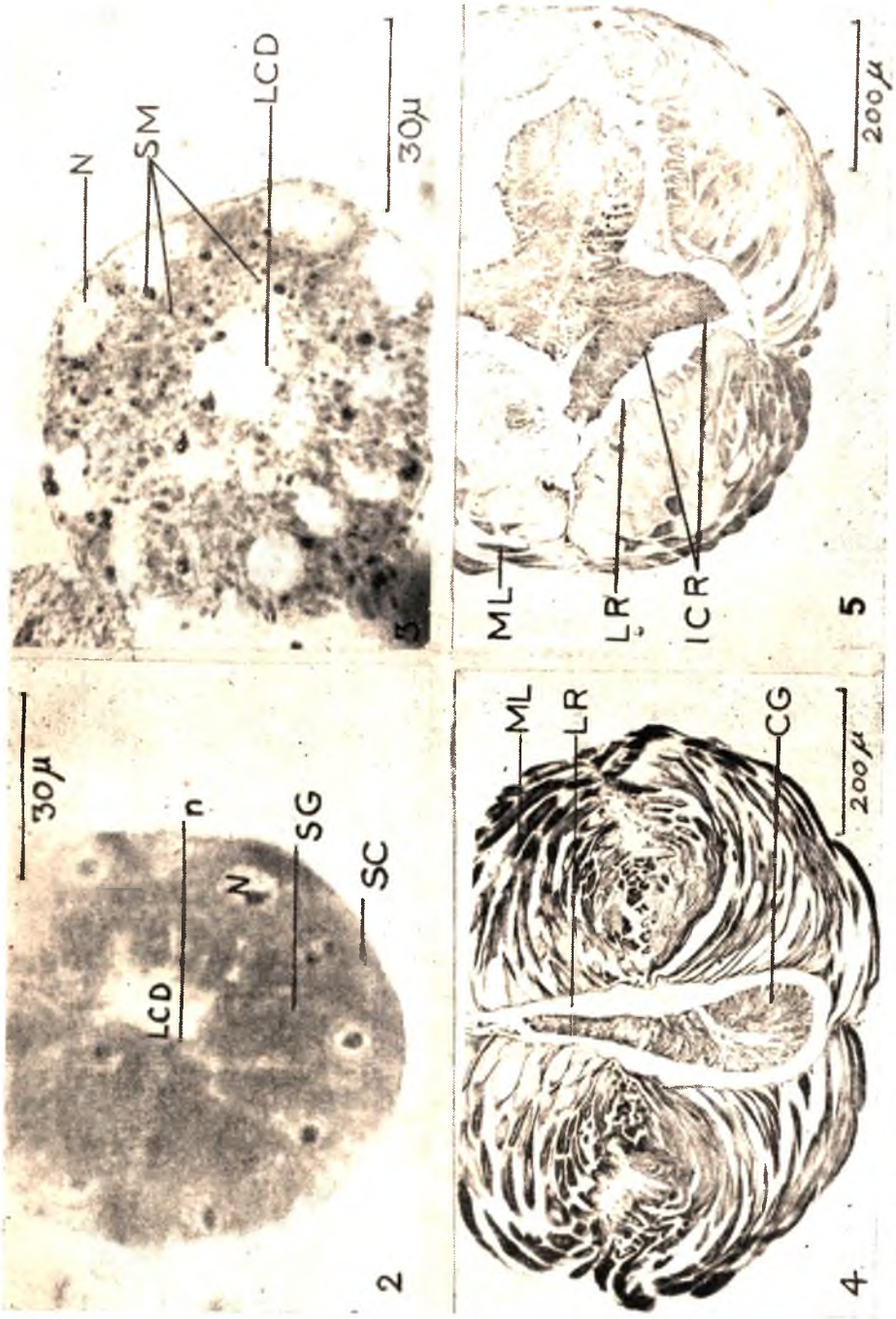

Fig. 2. T. S. through the acid gland showing disposition of secretory cells around the lumen of the collecting duct. LCD-lumen of collecting duct; N-nucleus of secretory cell; n-nucleus of chitogenous cell; SC-secretory cell; SG-secretory granules. Z/BPB. 3. T. S. through acid gland showing various types of sudanophilic bodies in the secretory cells; LCD-lumen of collecting duct; N-nucleus of secretory cell; SM-secretory material. FC/SBB. 4. T. S. through reservoir showing enclosed convoluted gland; CG-convoluted gland; LR-lumen of reservoir; ML-muscle layer, Z/BPB. 5. T. S. through middle region of reservoir and convoluted gland. ICR-intracellular reservoir; LR-lumen of reservoir; ML-muscle layer. Z/FF.

Cytochemical studies on the poison gland reveal interesting details of the chemistry of the cells and their secretion. The cytoplasm is basophilic as indicated by methyl green pyronin G (JORDAN & BAKER, 1955) technique. It is also rich in proteins demonstrable by mercuric bromphenol blue (MAZIA *et al.*, 1953) and ninhydrin Schiff (YASUMA & ITCHIKAWA, 1953) techniques. Carbohydrates are comparatively scarce as indicated by only faint postivity to periodic acid-Schiff (HOTCHKISS, 1948) test. Appreciable amount of lipids is demonstrated in the cytoplasm by Sudan black B (BAKER, 1949), acid haematein (BAKER, 1946) and Nile blue sulphate (CAIN, 1947) techniques.

Mercuric bromphenol blue shows a number of small secretion granules rich in proteins (Fig. 2). Ninhydrin-Schiff test specifies these to be mainly proteins with bound amino groups. The granules are usually concentrated towards the luminal end. A few small granules rich in proteins with sulfhydryl group can be seen dispersed in the cytoplasm after ferric-ferricyanide (CHEVREMONT & FREDERIC, 1943) test. The bulb-like reservoirs in the cells of the convoluted gland stain prominently after mercuric bromphenol blue and ferric-ferricyanide tests (Figs. 4 & 5). They also stain distinctly pink after Himes and Moriber's test (HIMES & MORIBER, 1956) indicating the presence of some polysaccharides in the secretion. Periodic acid-Schiff, followed by acetylation and KOH reversal techniques (MCMANUS & CASON 1950), stains only a few small granules in the wedge-shaped secretory cells. Best's carmine method (BEST, 1906) also does not give a definitive reaction. The cuticular lining of the central collecting canal of the free filaments as well as that of the intracellular reservoirs and the convoluted collecting duct stains distinctly with Alcian blue technique (STEEDMAN, 1950) showing an abundance of acid mucosubstances. Sudan black B technique employed for the demonstration of lipids in general shows secretory material of different forms viz., (i) small irregular masses of lipoidal secretion, (ii) variedly sized secretion granules; (iii) small membrane enclosed bodies; and (iv) a few small hyaline, unstained vesicles (Fig. 3). Acid haematein (followed by its pyridine extraction control) and Nile blue sulphate techniques further confirm the presence of phospholipids in these inclusions. The cells of the convoluted gland generally pick up more stain than those of the free filaments.

DISCUSSION

Evidently the wedge-shaped secretory cells of the free poison gland filaments and the small, polygonal cells of the convoluted poison gland of *Polistes hebraeus* produce a venomous secretion which is stored in the reservoir and ejected through the sting *via* the ejaculatory duct when needed. The two free filaments in *P. hebraeus* arise anteriorly from the reservoir, they then curve outwards and backwards to come to lie on the sides of the reservoir near its base unlike the condition found in the various families of ants (HERMANN, 1968 a, b, 1969 a; HERMANN & BLUM, 1966, 1967, 1968; CALLAHAN *et al.*, 1959), where the two filaments leave the reservoir laterally near its distal end. In *Nasonia vitripennis* RATCLIFFE & KING, 1969) there is only a single acid gland filament that leaves the reservoir proximally from its dorsal wall. The single acid gland filament of *Apis dorsata*, however, opens anteriorly into the reservoir (KANWAR & BRAR, 1976 a). The condition of the acid gland

filaments described for *Vespa orientalis* (KANWAR & SETHI, 1971) is similar to what has been found in *P. hebraeus*. The convoluted gland observed enclosed in the lumen of the reservoir corresponds to similar structure described in various genera of ants CALLAHAN *et al.*, 1959; HERMANN & BLUM, 1966, 1967), the enclosing reservoir in the ants however, lacks the strong and well developed muscles observed in *Polistes hebraeus*. The reservoirs of *Nosonia vitripennis* (RATCLIFFE & KING, 1969), *Vespa orientalis* (KANWAR & KANWAR, 1975) and *Apis dorsata* (KANWAR & BRAR, 1976 a), however, differ in lacking the convoluted gland.

The wedge-shaped secretory cells of the free-filaments, the small polygonal cells of the convoluted gland, have their counterparts in the other hymenopterans also. The chitogenous cells found appressed to the lumen in the free poison gland filaments of *P. hebraeus* are similar to those described in the cockroach colleterial gland (MERCER & BRUNET, 1959). (RATCLIFFE & KING, 1969) and LAWRENCE & STADDON (1975) have observed similar cells pressed against the lumen and are of the opinion that these are cuticle secreting. The bulb-like reservoirs observed in the polygonal cells of the convoluted gland have also been described in *Solenopsis saevissima* (CALLAHAN *et al.*, 1959). The authors have further mentioned the presence of fine ducts that connect these reservoirs to the convoluted collecting duct.

Very few reports on the cytochemical make-up of the poison gland in hymenopterans are available. The strong positivity for proteins observed in the cytoplasm and secretory granules of the poison secreting cells of *Polistes hebraeus* has been noticed in some other hymenopterans also. RATCLIFFE & KING (1969), during their studies on *Nasonia vitripennis* observed that the venom within the reservoir lumen stains more strongly with ninhydrin Schiff reaction than the contents of the acid gland lumen and opined that this suggests the addition of proteinaceous material to the final secretory products, from a source other than the cytoplasm of the acid gland. The generally stronger positivity of the cells of the convoluted gland in *P. hebraeus* suggests that these might be the source of the additional proteins in this animal at least. The variedly sized secretory granules observed in *P. hebraeus* specially with lipid stains are comparable to the dark bodies in the venom cells of *Apis dorsata* (KANWAR & BRAR, 1976 b), to the cytochemically complex secretory globules of *Vespa orientalis* (KANWAR & SETHI, 1971) and to the electron dense bodies described by EISNER *et al.* (1964). RATCLIFFE & KING (1969) have associated the abundance of lipids in the cytoplasm of the acid gland of *N. vitripennis* with the membranes of the extensive endoplasmic reticulum. The few small vacuoles observed in *P. hebraeus* may represent sites of water diffusible components leached out during aqueous fixations.

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TWO NEW AND TWO KNOWN SPECIES OF *DROSOPHILA* FROM RIMBICK, WEST BENGAL, INDIA

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Taxonomic account of four species of *Drosophila* is given. Two of them, *D. paralongifera* and *D. neomakinoi* are described as new, while *D. pentastriata* Okada and *D. acutissima* Okada are recorded for the first time from India.

(*Key words:* new *Drosophila*)

Accompanying the development of genetical and evolutionary knowledge in the genus *Drosophila*, taxonomic studies in this genus have also taken rapid strides during last few years and accumulated considerable data on the species inhabiting the subcontinent of India (See the review of Gupta, 1974 and other recent papers; Dwivedi, 1979; Dwivedi and Gupta 1980; Gupta and Dwivedi, 1980; Gupta and Singh, 1977, 1979; Prakash and Reddy, 1977, 1978, 1979 a, 1979 b; Sajjan and Krishnamurthy, 1975; Sajjan and Reddy, 1975; Singh and Gupta, 1977 a, 1977 b, 1980). These studies have indicated that the members of the genus *Drosophila* are fairly distributed throughout the subcontinent of India. However, in view the great size of the country and its considerable variety of habitats it is believed that there are undoubtedly more species awaiting discovery. The present paper embodies the results of several surveys undertaken at and around Rimbick.

Material for the present study was collected from Rimbick and its surrounding areas in the month of September 1979. Rimbick, a wild hilly area in Darjeeling district, West Bengal, India is located at an elevation of about 7500 feet above sea level. The area is characterized by having dense evergreen, coniferous forest covering medium to very steep slopes and extremely moist condition due to heavy rainfall. The flies were collected both by using different fermenting fruits as baits and also by net-sweeping over fallen flowers, fruits and wild vegetation.

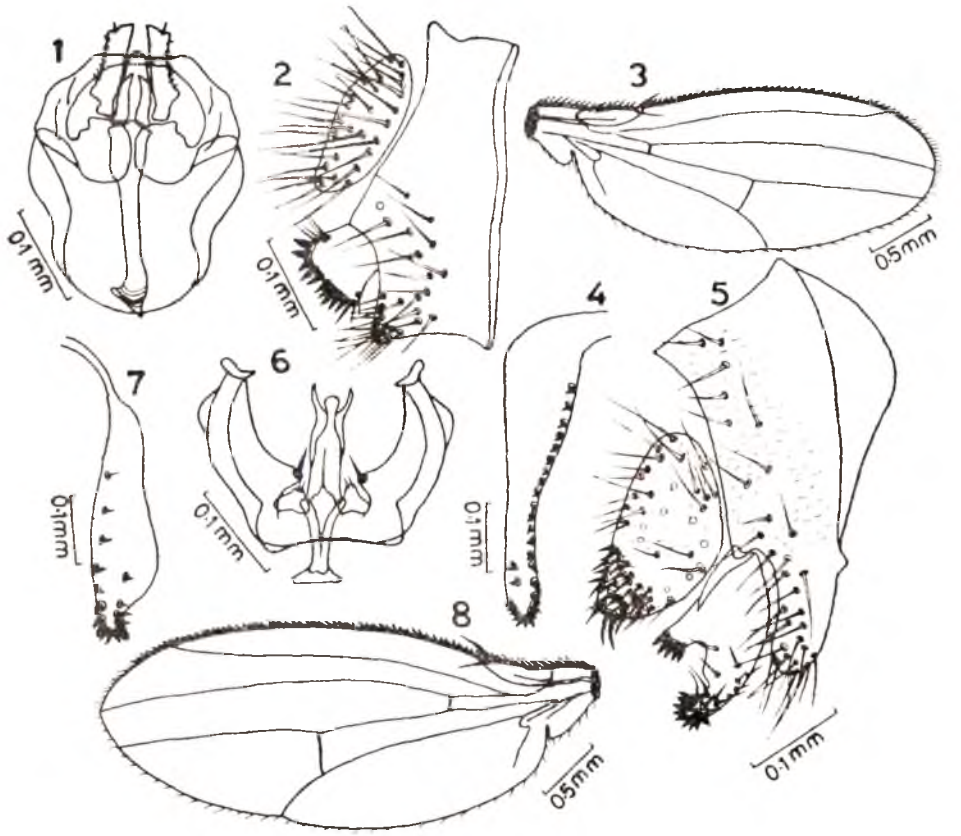
Genus *Drosophila* Fallen

Drosophila Fallen, 1823, Diptera Sueciae Geomyz., 2:4. Type species: *Musca funebris* Fabricious; Sweden.

1. Drosophila (Drosophila) paralongifera sp. nov.

Body length: 2.34 mm (♂); 2.75 mm (♀).

Head, ♂ and ♀: Arista with 2 branches above and 1 below in addition to terminal fork. Antennae with second segment brown; third segment yellowish brown. Frons brownish black, lighter anteriorly. Ocellar triangle black. Orbitals in ratio 8:5:7. Second oral not differentiated, vibrissa long and prominent. Palpi pale



Figs. 1—4: *Drosophila paralongifera* sp. nov. : 1—phallic organs; 2—periphallic organs; 3—male wing; 4—egg-guide. Figs. 5—8: *Drosophila neomakinoi* sp. nov. : 5—periphalic organs; 6—phallic organs; 7—egg-guide; 8—male wing.

brown, with one stout apical and 3—4 fine marginal setae. Carina brown, narrow and low. Face and cheek brown, greatest width of cheek 1/9 greatest diameter of eye. Postvertical moderate in size. Ocellars long. Clypeus dark brown. Eyes dark red.

Thorax, ♂ and ♀ : Acrostichal hairs regular, in eight rows between dorsocentrals. Anterior scutellars divergent; posterior scutellars crossed. Distance between anterior and posterior dorsocentrals one-third distance between anterior pairs. Mesonotum dark brown, much lighter near humeral region. Scutellum brown, lighter at margin. Thoracic pleura dark brown. Sterno-index about 0.5.

Legs dull yellow, each with last tarsal segment brown, preapicals on all three tibiae; apicals on first and second tibiae.

Wings ♂ and ♀ (Fig. 3) Dusky. Length about 2.64 mm. Approximate indices: C-index 2.78; 4V-index 1.5; 4C-index 0.78; 5X-index 1.34. Two equal setae at the apex of first costal section; heavy setae on about basal 2/7 of third costal section. Halteres white.

Abdomen, ♂ and ♀: Abdominal tergites shiny yellow, 1-2T with broadly interrupted and the remainder tergites with mildly interrupted broad black bands.

Periphallic organs (Fig. 2): Epandrium dark brown, broad, truncate below, with upper portion bare and lower portion with about 28 long setae. Surstylus quadrate, distal margin broad, with ten pointed teeth on entire margin, and a few fine setae on either side of the teeth. Cerci oblong, yellowish brown, separated from genital arch and with 25 long setae.

Phallic organs (Fig. 1): Aedeagus yellowish orange, short and basally with two apically pointed curved processes. Basal apodeme of aedeagus twice as long as aedeagus. Anterior gonopophyses brown, elongate, basally contiguous with aedeagus and with a row of 7-8 sensilla along outer margin. Posterior gonopophyses yellowish brown, large and fused together giving an arch-like appearance. Caudal margin of hypandrium without submedian spines. Ventral fragma weakly quadrate.

Egg-guides (Fig. 4): Lobe yellowish brown, narrowing distally, with 26 small marginal and 2 discal black teeth. Basal isthmus broad and short.

Holotype ♂, INDIA: WEST BENGAL, Rimbick, Darjeeling district, September 1979 (Gupta and Singh). **Paratypes:** 1 ♂, 1♀ same locality and collectors as holotype. Deposited in Museum of Department of Zoology, Banaras Hindu University, Varanasi.

Relationships: D. paralongifera a member of the subgenus *Drosophila* appears to be an unique species in having elongate anterior gonopophyses with a row of 7-8 sensilla along outer margin, posterior gonopophyses large and fused together giving an arch like appearance, surstylus quadrate, with ten pointed teeth on entire margin and its unusually long egg-guide. It superficially resembles *D. trizonata* Okada (member of the *bizonata* species group) in having fused posterior gonopophyses, but differs remarkably in several other important taxonomic characters.

Distribution: India.

2. Drosophila (Drosophila) neomakinoi sp. nov.

Body length: 2.85 mm (♂): 3.02 mm (♀).

Head, ♂ and ♀: Arista with 3-4 minute branches above and 1 below in addition to terminal fork. Antennae with second segment dark brown; third segment pale brown. Frons tanish brown, ocellar triangle brownish black. Orbitals in ratio 5:3:7. Vibrissa well developed large and stout; second oral not differentiated. Palpi orange, with one prominent apical and one marginal setae. Carina yellowish brown, narrow and high. Face and cheek yellowish brown, greatest width of cheek 1/5 greatest diameter of eye. Postvertical long. Ocellars long inserted well outside ocellar triangle. Clypeus brown. Eyes dark red.

Thorax, ♂ and ♀: Acrostichal hairs regular, in six rows between dorsocentrals. Anterior scutellars convergent: posterior scutellars crossed. Distance between anterior and posterior dorsocentrals half distance between anterior pairs. Mesonotum and scutellum unicolorous, shiny yellow to yellowish brown. Thoracic pleura yellow. Sterno-index about 0.7.

Legs pale yellow, preapicals on all three tibiae: apicals on first and second tibiae.

Wings ♂ and ♀ (Fig. 8): Hyaline. Length 2.16 mm. Approximate indices: C-index 2.97; 4V-index 1.54; 4C-index 0.76; 5X-index 1.59. Two subequal setae at the apex of first costal section; heavy setae on

about basal 2/5 of third costal section. Halteres whitish yellow.

Abdomen ♂ and ♀: Abdominal tergite shiny yellow, with medially interrupted and laterally projected apical black bands.

Periphallic organs (Fig. 5): Epandrinm brownish black, pubescent, broadened dorsally and narrowly projected below, upper portion with 8 long setae; lower portion with about 35 similar setae. Surstylus brown, large, apically divided into two, lower part with 11-13 stout, black teeth and 10-12 thick setae; upper part smaller, with 5-6 thick and pointed teeth. Cerci brown, oval, pubescent, with 32 setae and 28 pointed short teeth ventrally.

Phallic organs (Fig. 6): Aedeagus pale yellow, rod shaped, apically with lateral processes. Basal apodeme of aedeagus nearly equal. Anterior gonopopyhyses small, conical, each with two apical minute sensilla. Posterior gonopophyses obscure. Caudal margin of the hypandrium with a pair of small submedian spines. Ventral fragma quadrate.

Egg-guides (Fig. 7): Lobe yellowish brown, apically narrowly rounded, with 13 marginal and 4 discal teeth. Basal isthmus long and narrow.

Holotype ♂, INDIA: WEST BENGAL, Rimbick, Darjeeling district, September 1979 (Gupta and Singh). **Paratypes:** 1 ♂, 3 ♀♀, same locality and collectors as holotype. Deposited in the Department of Zoology, Banaras Hindu University, Varanasi, India, and Department of Biology, Tokyo Metropolitan University, Tokyo, Japan.

Relationships: The characteristic features of its periphallic organs suggests its inclusion in the *melanderi* species group. Where it closely resembles *D. makinoi* Okada in having identical pattern of periphallic and phallic organs, but distinctly differs from it in having 11-13 stout black teeth on lower part of surstylus (numerous recurved setae in *makinoi*), cerci with about 28 thick pointed teeth ventrally (20 short but strong setae in *makinoi*), and in many other taxonomical characters. However, *D. neomakinoi* also resembles slightly *D. cameraria* Haliday (= *pallida* Zetterstedt) superficially, but it differs drastically from it in male genitalia. The other members of the *melanderi* group, *D. melanderi* Sturtevant, *D. magnafumosa* Stalker and Spencer and *D. ordinaria* Coquillet have no significant resemblance with *D. neomakinoi*.

Distribution : India.

3. Drosophila (Drosophila) pentastriata Okada

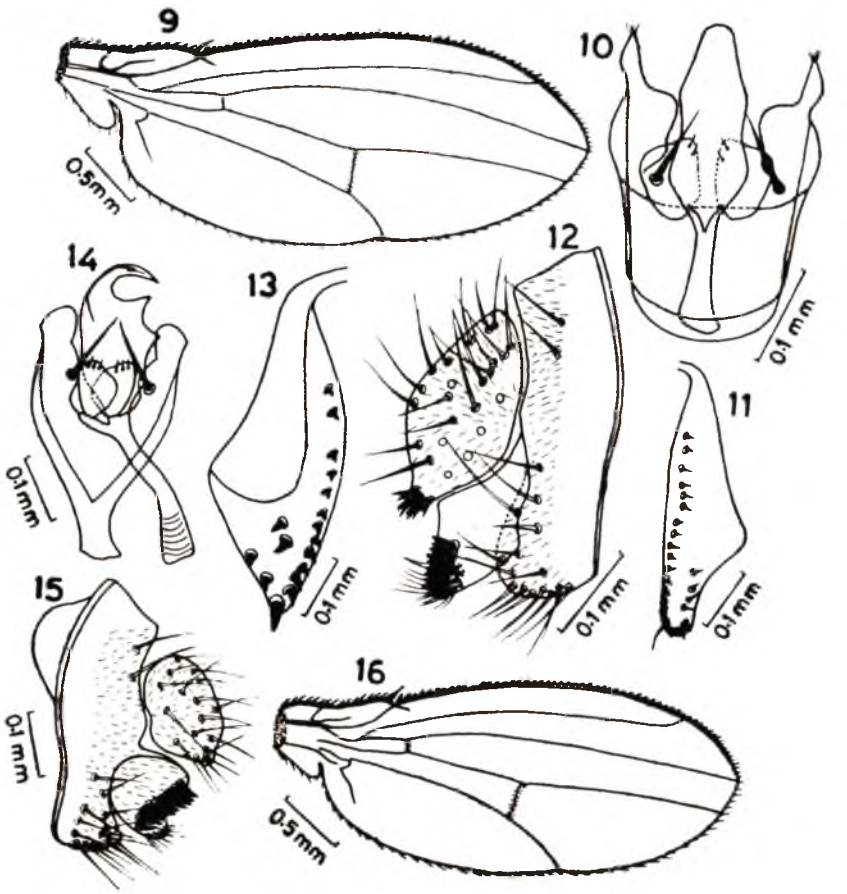
D. pentastriata Okada, 1966, Bull. Brit. Mus. (Nat. Hist.) Ent. Suppl. 6: 109.

Head, ♂ and ♀: Palpi yellowish brown, with one prominent apical and one marginal setae. Orbitals in ratio 7:3:11.

Thorax, ♂ and ♀: Acrostichal hairs regular, in six rows between dorsocentrals. Scutellum reddish yellow, with two longitudinal dark brown stripes.

Wings, ♂ and ♀: (Fig. 9): Clear posterior cross vein mildly fuscous. Approximate indices: C-index 3.44; 4V-index 1.56; 4C-index 0.61: 5X-index 1.15. Other details as described by Okada (1966).

Periphallic organs (Fig. 12): Epandrium yellowish brown, pubescent, narrow, slightly swollen below, upper portion with 2 long setae; lower portion with 15 similar setae. Surstylus large, with 9-10 stout teeth and several ventral setae. Cerci



Figs. 9—12: *Drosophila pentastriata:* 9—male wing; 10—phallic organs; 11—egg-guide; 12—periphallic organs. Figs. 13—16: *Drosophila acutissima:* 13—egg-guide; 14—phallic organs; 15—periphallic organs; 16—male wing.

large, pubescent, with about 25 long setae, and a tuft of few small black setae ventrally.

Phallic organs (Fig. 10): Posterior gonopophyses obscure. Caudal margin of hypandrium with a pair of long spines. Ventral fragma quadrate. Other details as described by Okada (1966).

Egg-guides (Fig. 11): Lobe yellowish brown, medially swollen, apically narrow and rounded, with 21 marginal and 4 discal teeth. Basal isthmus short and narrow.

Specimens examined: India: West Bengal, Rimbick, Darjeeling district 3 ♂♂, 4 ♀♀, September 1979.

Distribution: Nepal and India (new record).

4. Drosophila (Drosophila) acutissima Okada

Drosophila acutissima Okada, 1956, Syst. Study Dros. Japan: 139.

Head, ♂ and ♀: Arista with 4 branches above and 2 below in addition to terminal

fork. Orbitals in ratio 7:3:11. Second oral thin about one third of vibrissa. Cheek yellowish brown, greatest width of cheek 1/5 greatest diameter of eye.

Thorax, ♂ *and* ♀: Acrostichal hairs regular, in six rows between dorsocentrals. Distance from anterior dorsocentral to posterior dorsocentral 3/7 distance between two anterior dorsocentrals.

Wings, ♂ and ♀ (Fig. 16): Clear, cross veins mildly fuscous. Approximate indices: C-index 3.5; 4V-index 1.88; 4C-index 0.72; 5X-index 2.11. Other details as described by Okada (1956).

Periphallic organs (Fig. 15): Epandrium pubescent, upper portion with 2; lower portion with 16-18 setae. Surstylus oval, apical margin concave, with a row of 10 pointed teeth, and a few ventral setae. Cerci brown, somewhat oval and with 16 setae.

Phallic organs (Fig. 14): Aedeagus pale brown, medioventrally swollen, apically hooked. Basal apodeme of aedeagus slightly longer than aedeagus. Anterior gonopophyses oval, with 2-3 minute apical sensilla. Posterior gonopophyses obscure. Caudal margin of hypandrium with a pair of submedian spines. Ventral fragma dark brown, Y-shaped, marginally black.

Egg-guides (Fig. 13): Lobe orange brown, medially broad, apically conical, with 15 marginal and 2 discal teeth, apical tooth largest. Basal isthmus narrow and long.

Specimens examined: India: West Bengal, Rimbick, Darjeeling disirict. 4 ♂♂; 11 ♀♀ September 1979.

Distribution: Japan, Nepal and India (new record).

Remarks: The Indian strain of *D. acutissima* strictly resembles the Nepalese form in having medioventrally swollen aedeagus, but differs from the original Japanese form in not having distinct lateral lobe of aedeagus.

Acknowledgements:—The authors wish to express their sincere gratitude to Dr. T. Okada, Emeritus Professor, Department of Biology, Tokyo Metropolitan University, Tokyo, Japan for extending his help in confirming the identifications. Thanks are also due to Prof. J. P. Thapliyal, Head of the Zoology Department for providing laboratory facilities and to the University Grants Commission for financial support.

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TWO NEW SPECIES OF *AGNESIELLA* (*DRABERIELLA*) (HOMOPTERA: CICADELLIDAE : TYPHLOCYBINAE) FROM JAMMU AND KASHMIR INFESTING *ALNUS NITIDA*

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Two new species of the subgenus *Draberiella* of the genus *Agnesiella* namely, *jammuensis* and *alni* from Kudh (Jammu and Kashmir) collected from *Alnus nitida* are described and illustrated. A Key to the known species of this subgenus is also given. *Sarejuia* Ghauri is treated as a junior synonym of *Draberiella* Dworakowska.

(*Key words: Draberiella, Agnesiella,* Cicadellidae new species)

Dworakowska (1970) described the genus *Agnesiella* (type species : *Typhlocyba aino* Matsumura) and distinguished it from *Linnavouriana* Dlabola. Later she (1971) erected the subgenus *Draberiella* with *Chikkaballapura quinquemaculata* Distant (1918) as its type species. Unaware of this, Ghauri (1974), however, described the genus *Sarejuia* for *C. quinquemaculata* and also included his new species *decorata* in it. Ahmed (1970) described the *quinquemaculata* under the genus *Typhlocyba*. His material came from Garhi Habibullah, Pakistan collected on *Alnus nitida*. However, the male genitalia illustrations of Ahmed (1970) differed significantly from those illustrated by Dworakowska (1971) which are based on the holotype, suggesting misidentification by Ahmed.

Sarejuia Ghauri, therefore, is treated here as a junior synonym of *Draberiella*. Two species of the subgenus collected by the authors in Kudh (Jammu & Kashmir) on *A. nitida* plant are described here as new. A key to the ten known species of *Draberiella* is also provided for easy recognition. The holotypes of the new species are deposited in the Department of Biosciences, University of Jammu, Jammu and the paratypes in the Zoological Survey of India, Calcutta.

1. Agnesiella (Draberiella) jammuensis sp. nov. (Figs. 1—10).

Colour: Pale yellow ochraceous, with black spots as follows: two rounded spots on vertex, three spots on pronotum, two triangular basal spots on scutellum. Eyes black. Two prominent spots in the anterior half, on the costal margin black and transverse suffusions on forewings brownish black. Hindwing smoky-white.

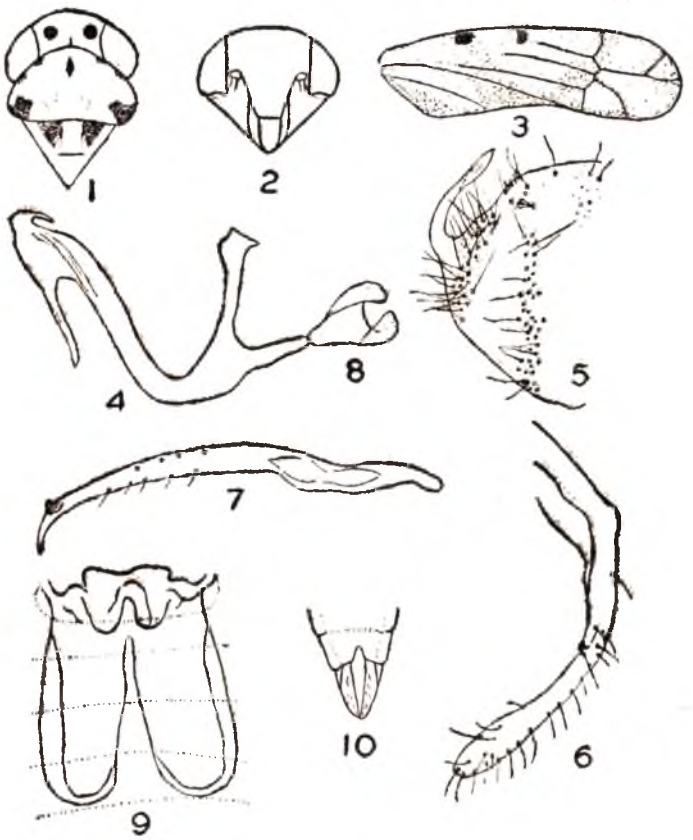
External features: Macropterous vertex slightly more than half the length of pronotum, produced little in front of eyes: breadth between eyes slightly lesser than double the median length. Head including eyes slightly broader than the pronotum. Scutellum much broader basally than its median length, 2/3 the length of pronotum. Hind femoral spinulation 2, 1, 1.

Forewings (Fig. 3) rather long, slightly narrowing towards apex: first apical cell short with the basal limiting vein arched, second cell widest towards the apex; third

petiolate, subtriangular, petiole longer than the length of the third cell. Hindwings narrowing apically, submarginal vein confluent with the apex of Cu_2; with two apical cells, apically open.

Male genitalia: Subgenital plate (Fig. 6) long, pigmented, tooth present at almost 2/3 the length from base, apical 1/3 beset with setae. Pygofer (Fig. 5) rounded apically, with a long appendage at the posterior margin directed upwards, posterior margin beset with two groups of hairy setae. Paramere (Fig. 7) apically pointed with a small preapical lobe; beset with setae in the preapical region on the outer margin. Connective (Fig. 8) broad proximally, with the sides rolled, then abruptly narrowing in the mid region. Aedeagus (Fig. 4) with well developed basally arising dorsal apodeme, shaft curving dorsad; a long preapical horn-like process on the posterior side directed ventrad: and two hook-like denticules apically; gonopore dorsad, slightly preapical. Abdominal apodemes (Fig. 9) well developed.

Female genitalia: 7th sternum (Fig. 10) concave marginally and then deeply notched medially. Ovipostor and pygofer apices at the same level.



Figs. 1—10: *Agnesiella (Draberiella) jammuensis* sp. nov. 1—head and thorax; 2—face; 3—forewing; 4—aedeagus, lateral view; 5—pygofer; 6—male subgenital plate; 7—paramere; 8—connective; 9—abdominal apodemes; 10—female genitalia.

Measurements: (In millimeters, female in parentheses). Length with wings 3.2 (3.45); length without wings 2.2 (2.45); vertex 0.2/0.4 (0.225/0.4): pronotum 0.45/0.85 (0.5/0.85): scutellum 0.35/0.6 (0.45/0.65); (length breadth).

Holotype: ♂ with abdomen on slide from INDIA : JAMMU & KASHMIR, Kudh, 10. ix, 1973, ex *Alnus nitida*, B. Sharma Coll.

Paratypes: 5 ♂♂ and 12 ♀♀ with same data as the holotype.

Remarks: Of all the species described under *Agnesiella (Draberiella), jammuensis* sp. nov. resembles in many characters with the unnamed species of Ahmed (1970) who misidentified it as *Typhlocyba quinquemaculata*. But it can be distinguished by (1) the absence of any median lobe on cephalic margin of connective: (2) process of the aedeagal shaft in *jammuensis* arises more caudal compared to Ahmed's species where it arises almost from the middle of the shaft; (3) petiole of the 3rd apical cell of forewing is longer than the cell itself in *jammuensis* while it is shorter in the figures of the forewing which have been given by Ahmed for his species; (4) posterior margin of pygofer in *jammuensis* is pointed (towards the base of the apical process) while it is truncated in Ahmed's species.

2. Agnesiella (Draberiella) alni sp. nov. (Figs. 11—20)

Colour: Colour in male like in preceding species, but two additional spots on passage from vertex to frons and two on pronotum black. Sexes show striking colour dimorphism. Females in addition to costal black spots and brownish-black markings on forewing have reddish colour extending from costal margin to claval suture in pre-apical and basal region of the forewing (shaded in squares in Fig. 13). Veins of hind wing brownish-red. Besides vertex, pronotum and scutellum may be tinged with red. Males lack reddish markings.

External features: Macropterous, vertex longer than 1/3 the length of pronotum; breadth double the median length. Head slightly broader than pronotum. Scutellum, at base, broader than long; slightly longer than 1/3 the length of pronotum. Hind femoral chaetotaxy 2, 1, 1.

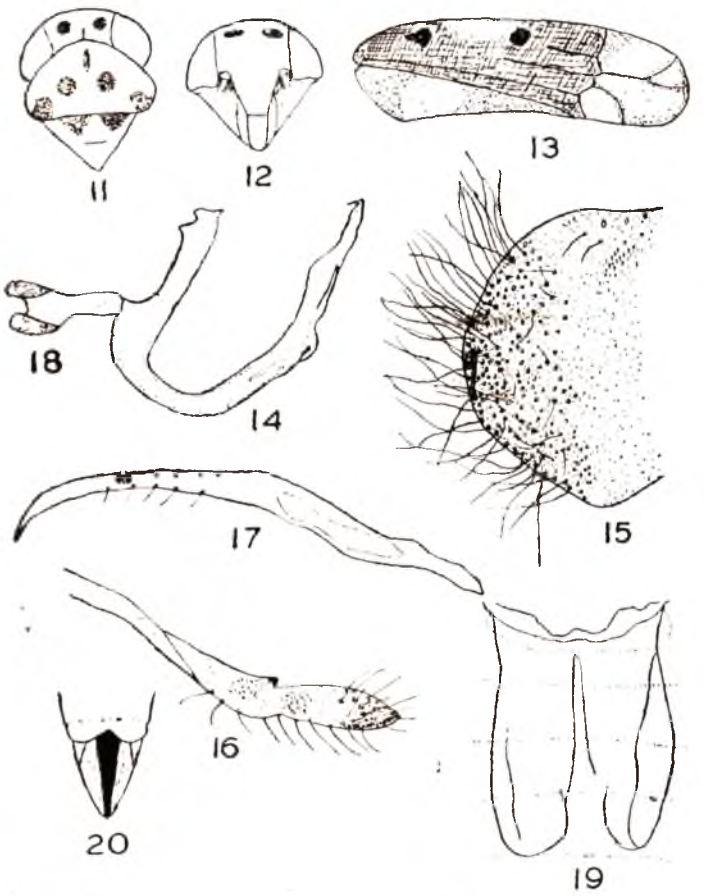
Forewing and hindwing venation as *jammuensis* sp. nov.

Male genitalia: Subgenital plate (Fig. 19) long and narrow, with a pigmented tooth at 2/3 the length from base and a few setae at apex and outer margin in apical 1/3. Pygofer (Fig. 15) Caudally rounded, its margin entire, with a small appendage; its posteriad beset with marginal and submarginal hairly setae. Paramere (Fig. 17) pointed apically and curved laterad at apex, with a small lobe, much proximad than in *jammuensis:* preapically beset with setae on the outer margin. Connective as in *jammuensis* (Fig. 18). Aedeagal shaft (Fig. 14) much curved, with a preapical posteriorly directed and an anteriorly directed process and with a terminal hook-like process; gonopore terminal. Abdominal apodemes (Fig. 19) well developed.

Female genitalia: 7th sternum (Fig. 20) notched medially. Ovipositor black; pygofer lobes reaching the apex of the ovipositor.

Measurements: (In millimeters, female in parentheses). Length with wings 3.2 (3.45); length without wings 2.2 (2.45): vertex 0.2/0.4 (0.225/0.4); pronotum 0.45/0.85 (0.5/0.85): scutellum: 0.35/0.6 (0.45/0.65).

Holotype: ♂ with abdomen on slide from INDIA: JAMMU & KASHMIR, Kudh: 10. ix. 1973, ex *Alnus nitida;* B. Sharma coll.



Figs. 11—20: *Agnesiella (Draberiella) alni* sp. nov. 11—head and thorax; 12—face; 13—forewing, female; 14—aedeagus, lateral view; 15—pygofer; 16—male subgenital plate; 17—paramere; 18—connective; 19—abdominal apodemes; 20—female genitalia.

Paratypes: 1 ♂ with abdomen on slide and 10 ♀♀ with same data as for the holotype.

Remarks: *Agnesiella (Draberiella) alni* sp. nov. can be differentiated from all the species described so far under the subgenus by the absence of prominent process at the posterior margin of pygofer, distinctive colour markings on head and thorax and differences in colour in males and females. In general shape of the aedeagus the new species resembles *Agnesiella (Draberiella) irma* Dworakowska but the processes of the aedeagal shafts are distinctive in the two species.

KEY TO THE KNOWN SPECIES OF *AGNESIELLA (DRABERIELLA)*

1. Pygofer with two pointed processes arising from posterior margin; aedeagus without pointed pre-apical spine-like process (Vietnam) ..... *lidia* Dworakowska

—Pygofer with one process arising at its posterior margin; aedeagus with pre-apical spine-like processes 2

2. Aedeagal processes arising near base of the curvature of shaft 3

—Aedeagal processes arising far beyond the curvature of shaft 4

3. Aedeagus with one slender undivided apically directed process (Vietnam) ..... *ogromna* Dworakowska

—Aedeagus with one stout process further divided into three (two directed apically and one basally) (Vietnam) ..... *irma* Dworakowska

4. Two preapical processes arising independently from the aedeagal shaft 5

—One preapical process arising from aedeagal shaft 6

5. Both the processes directed apically; apex of aedeagal shaft blunt (Vietnam) ..... *olena* Dworakowska

—One of the processes directed apically while the other directed ventrally; apex of aedeagal shaft pointed (J & K, India) ..... *alni* sp. nov.

6. Process of aedeagus simple, not divided into two branches 7

—Process of aedeagus divided into two branches one directed caudad while the other directed cephalad (Vietnam) ..... *ela* Dworakowska

7. Aedeagal process directed towards apex (India) ..... *decorata* (Ghauri) Comb. nov.

—Aedeagal process directed towards the base of the shaft 8

8. Process of the aedeagus very small; pygoferal process directed caudad (India) ..... *quinquemaculata* (Distant)

—Process of aedeagus very long, pygoferal process directed upwards (dorsad) 9

9. Cephalic margin of connective with a median lobe, aedeagus with one dorsal terminal hook (Pakistan) ..... Unnamed (Ahmed, 1970)

—Cephalic margin of connective without a median lobe, aedeagus with two dorsal terminal hooks (J & K, India) ..... *jammuensis* sp. nov

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SOME NEW SPECIES AND HITHERTO UNKNOWN MORPHS OF APHIDS (HOMPTERA : APHIDIDAE) FROM HIMACHAL PRADESH, NORTHWEST INDIA

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Two new species, viz., *Aulacorthum delphinae* and *Dactynotus minatii* and four hitherto unknown morphs, viz., apterous oviparous female of *Aphis paraverbasci* Chakrabarti, alate male and apterous oviparous female of *Chaitophorus indicus* Ghosh, Ghosh and Raychaudhuri, alate male of *Diphorodon cannabis* (Passerini) and apterous oviparous female of *Nippolachnus* sp. of aphids are described from Himachal Pradesh, Northwest India.

(*Key words:* Aphid taxonomy, new species, unknown morphs)

Since 1973 systematic survey for aphids has been undertaken in the state of Himachal Pradesh, northwest India. Aphid samples so far examined has revealed the existence of 2 new species, viz. *Aulacorthum delphiniae*, *Dactynotus minatii* and 4 hitherto unknown morphs, viz. apterous oviparous female of *Aphis paraverbasci* Chakrabarti, alate male and apterous oviparous female of *Chaitophorus indicus* Ghosh, Ghosh and Raychaudhuri, alate male of *Diphorodon cannabis* (Passerini) and apterous oviparous female of *Nippolachnus* sp., which are being described.

Materials are in the collection of Entomology Laboratory, Department of Zoology, Calcutta University.

1. Aulacorthum delphinae sp. nov. (Figs. 1A—F).

Apterous viviparous female: Body about 2.47—2.65 mm long with about 1.17—1.42 mm as its maximum width. Head (Fig. 1B) finely spinulose on venter, with well developed diverging lateral frontal tubercles: median frontal prominence low but distinct: dorsal cephalic hairs long on tuberculate bases, with blunt apices, about 1.0—1.30 × b.d. III. Antennae (Fig. 1E) pale brown, 6-segmented, about 1.09—1.10 × body: segment I slightly inwardly projected and smooth: segment II smooth: segment III smooth excepting a few imbrications near base, rest of the flagellum gradually imbricated apicad: segment III with protuberant 16—23 secondary rhinaria on basal 0.35—0.41 portion: hairs on segment III like those on cephalic dorsum, about 0.40—1.10 × b.d. III. Midthoracic furca sessile. Rostrum reaching hindcoxae: u.r.s. (Fig. 1C) with 5—9 secondary hairs about 1.0—1.10 × h. t. 2. Abdominal dorsum pale; dorsal hairs like those on cephalic dorsum, longest hair on anterior tergites being about 0.70—1.03 × b. d. III: 7th and 8th tergites with 5—6 and 2—4 hairs respectively, longest of these being about 1.20—1.70, 1.0—1.60 b.d. III respectively. Siphunculi (Fig. 1D) long, cylindrical, strongly imbricated, with a few

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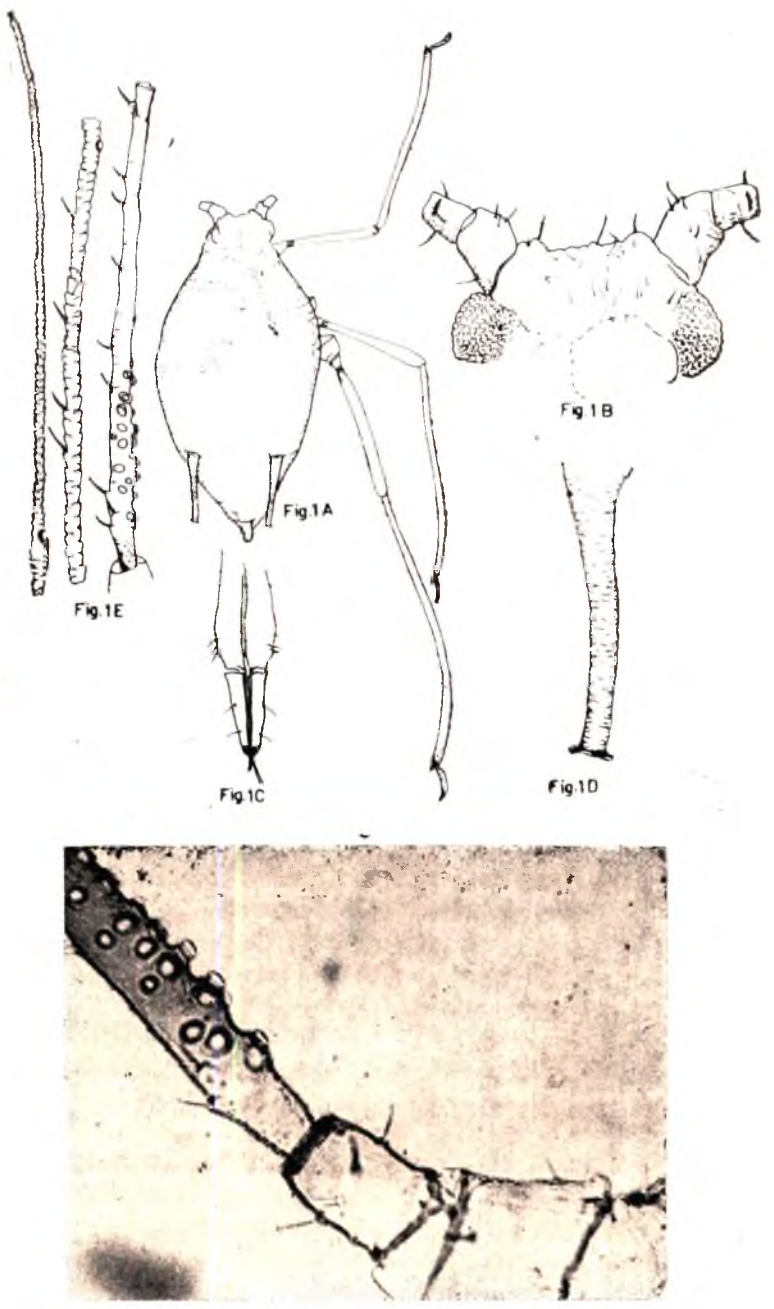


Fig. 1. *Aulacorthum delphiniae* sp. nov. : Aptera. A—Whole body; B—Head; C—U.r.s.; D—Siphunculus; E—Antenna.

preapical striae and a well developed cauda bearing 6—8 hairs. Legs normal, flange, about 2.0—2.50 × subpentagonal F. T. C. 3, 3, 3.

Measurements of the holotype in mm: Length of body 2.65, width 1.29: antenna 2.94, antennal segments III: IV: V: VI 0.85: 0.45: 0.40: 0.10 + 0.93: u. r. s 0.15: h. t. 2 0.15: siphunculus 0.54; cauda 0.22.

Collection data: **Holotype**: apterous viviparous ♀, INDIA: HIMACHAL PRADESH, Mashobra (*c* 2149 m), 22. vi. 79. from *Delphinium ajacis* (Ranunculaceae), coll. S. K. Das, **paratype:** 7 greenish apterous viviparous ♀♀ and 22 nymphs, collection data same as for holotype.

Remark: The present material cannot strictly be put in any known genus of Macrosiphini. But David *et. al.* (1970) described similar material as a new species under *Aulacorthum*. If their (op:cit.) idea is applied here then the present material can be considered at least for the time being under *Aulacorthum* and in that case the present material is treated as a new species resembling David's species. In future if some other similar species are found then a new genus may have to be erected when it should be characterised by the presence of low median frontal prominence, diverging lateral frontal tubercles, and protuberant secondary rhinaria in apterae viviparae.

2. Dactynotus minatii sp. nov. (Figs. 2A-F)

Apterous viviparous female : Body (Fig. 2A) pale brown, about 1.80—4.38 mm long with about 1.20—1.80 mm as its maximum width. Head pale, smooth, with

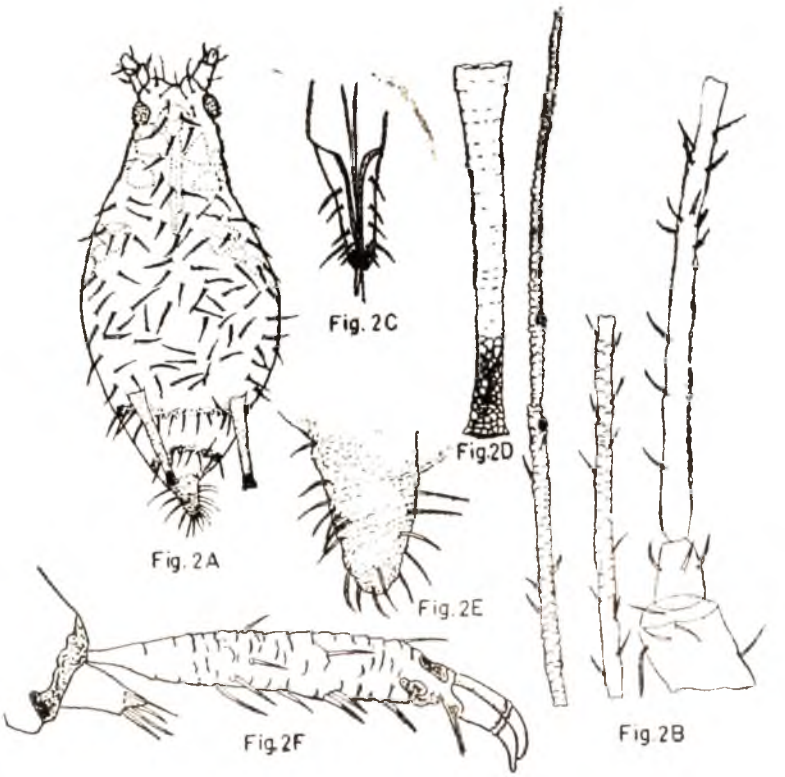


Fig. 2. *Dactynotus minatii* sp. nov.: Aptera. A—Whole body; B—Antenna; C—U.r.s.; D—Siphunculus; E—Cauda; F—Tarsi.

well developed, diverging lateral frontal prominence and a median frontal prominence; dorsal cephalic hairs long with acute apices. Antennae (Fig. 2B) 6-segmented, about 1.30—1.98 × body; flagellum gradually becoming imbricated apicad; p. t. about 5.26—5.92 × base of segment VI and about 0.66—0.81 × segment III; apterae with 12-20 strongly protuberant secondary rhinaria arranged in a row; primary rhinaria ciliated; hairs on flagellum with acute apices, longest hair on segment III about 1.07—1.72 × b. d. III. Midthoracic furca with a basal stem. Rostrum extending beyond midcoxae; u. r. s. (Fig. 2C) about 1.07—1.22 × h. t. 2 and bears 4—6 secondary hairs. Abdominal dorsum pale; dorsal hairs similar to those on cephalic dorsum; longest hair on anterior, 7th and 8th tergites about 2.42—3.0×, 2.07—3.83 × and 2.14—3.83× b. d. III respectively. Siphunculi (Fig. 2D) brown, cylindrical, reticulated on distal 0.12—0.22 portion, about 2.09—2.35× elongated cauda (Fig. 2E) bearing 11-16 hairs. Legs brown, dark at the apex; F. T. C. 4, 4, 4 (Fig. 2F).

Measurements of the holotype in mm: Length of body 2.14, width 1.42; antenna 4.84, antennal segments III: IV: V: VI 0.91: 0.75: 0.58: 0.18+1.15; u. r. s. 0.15; h. t. 20.13; siphunculus 0.69; cauda 0.33.

Collection data: Holotype: Apterous viviparous ♀, INDIA: HIMACHAL PRADESH, Kiarighat (*c* 1500 m), 17.x.79 from an unidentified plant, coll. S. K. Das, **paratypes**: 5 apterous viviparous ♀♀, Simla (*c* 2000 m) 22.x.79, from *Delphinium* sp. (Ranunculaceae); 1 apterous viviparous ♀ and many nymphs, Mashobra (*c* 2149 m), 22. vi. 79, from an unidentified plant of Ranunculaceae; 2 apterous viviparous ♀♀ and 1 nymph, Kufri (*c* 2633 m), 21. vi. 79, from an unidentified plant of Ranunculaceae, coll. S. K. Das.

Remark: The new species in having long body hairs and 4, 4, 4 tarsal chaetotaxy closely resembles *Uroleucon longisetosus* Chakrabarti and Verma, 1975 but can easily be distinguished by the following characters: 0.12—0.22 portion of siphunculi reticulated and siphunculi about 2.09—2.35 × cauda: 8th tergite with 6-8 hairs.

3. Aphis paraverbasci Chakrabarti (Figs. 3A—C)

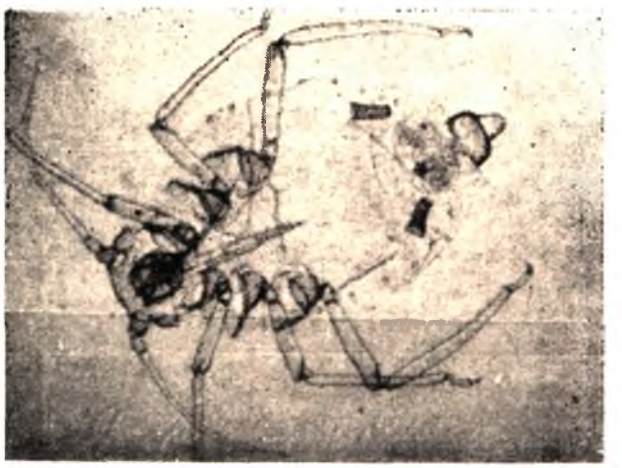
1976. *Aphis paraverbasci* Chakrabarti, Entomon, 1 (2): 171—173.

Apterous oviparous female: Body pale brown, about 1.41—1.57 mm long and about 0.76—0.90mm wide. Head smooth; dorsal cephalic hairs flagellate. Antennae 6-segmented: segments I and II dark and smooth; flagellum gradually imbricated apicad: p. t. about 1.70—2.0 × base of segment VI; hairs on segment III with acuminate apices, longest one being about 1.10—2.60 × b. d. III. Rostrum long and extending beyond hindcoxae; u.r.s. stiletto-shaped, about 1.90—2.10 × h. t. 2, bearing 2—3 secondary hairs. Abdominal dorsum pale bearing hairs with acuminate apices: longest hair on anterior, 7th and 8th tergites being about 3.60—4.50×, 3.30—5.90 × and 2.60—4.20 × b. d. III respectively. Siphunculi dark, cylindrical, imbricated, about 1.30—1.50 × cauda bearing 11—12 hairs. Hindtibiae (Fig. 3B) swollen with many pseudosensoria. F. T. C. 3, 3, 3. Other characters as in apterae viviparae.

Measurements of one apterous oviparous female in mm: Length of body 1.41, width 0.78; antenna 0.76 antennal segments III; IV: V: VI 0.16; 0.12: 0.10: 0.09+0.18; u. r. s. 0.18; h. t. 2 0.09: siphunculus 0.10; cauda 0.09.

Fig. 3A

Fig. 3B



Fig, 3. *Aphis paraverbasci* Chakrabarti : Apterous ovipara. A—Posterior portion of abdomee; B—Hind tibiae

Collection data: INDIA: HIMACHAL PRADESH: Manali (*c* 2050 m), 22.x.79.9 apterous viviparous ♀♀, 8 apterous oviparous ♀♀ and 3 nymphs from an unidentified host-plant, coll. S. K. Das.

Remark: Chakrabarti (1976) described the species from apterous viviparous female collected at SIMLA, HIMACHAL PRADESH. Find of apterous oviparous female at MANALI, HIMACHAL PRADESH suggests the possibility of completion of holocyclic mode of life-cycle in the region.

Fig. 4A

Fig. 5A

Fig. 4B

Fig. 4. *Chaitophorus indicus* Ghosh, Ghesh and Raychaudhuri : Apterous ovipara. A—Posterior portion of abdomen; B—Hind tibiae showing pseudosensoria.

4. Chaitophorus indicus (Figs. 4A —C and 5A,B)

1970. *Chaitophorus indicus* Ghosh, Ghosh and Raychaudhuri. Orient. Insects, 4(2): 196.

Apterous oviparous female:Head brown and smooth; dorsal cephalic hairs flagellate. Antennae 6-segmented, much shorter than body; flagellum gradually distinctly imbricated apicad; segment IV a little constricted at apical 0.25 portion; p. t. about 3.80× base of segment VI: primary rhinaria non-ciliated: flagellar hairs long with fine apices, longest one on segment III about 5.0—6.30×b. d. III. Rostrum reaching midcoxae: u. r. s. about 0.96—1.0 × h. t. 2 and bears 4 secondary hairs. Dorsum of abdomen pale with long fine hairs, longest one on anterior, 7th and 8th tergites about 7.0—8.30×, 7.30—9.10× and 6.0—8.30 × b. d. III. respectively. Siphunculi truncate, almost entirely reticulated, about 1.0 × knobbed cauda bearing 4 hairs. Subgenital plate as in Fig. 4A. Hindtibiae with many pseudosensoria (Fig. 4B).

Measurements of one apterous oviparous female in mm: Length of body 1.47, width 0.91; antenna 1.0. antennal segments III : IV:V:VI 0.21 :0.09 : 0.10 : 0.07+0.28: u. r. s. 0.11; h.t. 2 0.11: siphunculus 0.04; cauda 0.04.

Alate male: Body pale brown, elongate. Head brown, smooth on both surfaces and without frontal tubercles; dorsal cephalic hairs with acuminate apices. Antennae concolourous with head, 6 segmented, about 0.82—0.95 × body: segment III basally constricted; p. t. about 3.20—3.80× base of segment VI; segments III, IV & V with 19—22, 9—18 a d 5—7 irregularly arranged nonprotuberant secondary rhinaria respectively: primary rhinaria non-ciliated; hairs on flagellum with fine apices, longest one on segment III about 3.60—5.0×b. d. III. Rostrum reaching midcoxae; u. r. s. about 0.96—1.03 × h. t. 2 bearing 4 secondary hairs. Abdominal dorsum pale with segmentally arranged hair bearing spinopleural and marginal sclerotic patches: dorsal hairs long and with fine apices, longest one on anterior, 7th and 8th tergites about 6.0—7.50×,5.20—8.70× and 5.0—8.70 × b. d. III respectively. Siphunculi truncated reticulated over almost entire length. Cauda knobbed and bears 5—7 hairs. Male genitalia as in Fig. 5A. Legs normal. Wing venation normal. Other characters as in alate viviparous female.

Measurements of one alate male in mm: Length of body 1.18, width 0.46; antenna 0.97: antennal segments III: IV: V: VI 0.25: 0.16: 0.13: 0.07 + 0.24; u.r.s. 0.10; h.t. 2 0.10; siphunculus 0.06; cauda 0.03.

Collection data: INDIA: HIMACHAL PRADESH: Manali (c 2050 m), 28. x. 79, 1 apterous viviparous ♀, 3 apterous oviparous ♀♀, 5 alate ♂♂ and 6 nymphs from *Populus* sp. (Salicaceae), coll. S. K. Das.

Remark: Out of the chaitophorine genera known from India only two species of the genus *Periphyllus* are represented by sexuales (Chakrabarti, 1977). With the find of alate male and apterous oviparous female of *Chaitophorus indicus* Ghosh, Ghosh and Raychaudhuri two chaitophorine genera are now known to posses sexuales in Indian condition.

Note: Ghosh, Ghosh and Raychaudhuri (1970) while erecting the species *indicus* under the genus *Chaitophorous* Koch stated that ultimate rostral segment bears 8 hairs. Chakrabarti (1977) mentioned that *indicus* has 8 accessory hairs on ultimate rostral segment. Re-examination of the previously collected material reveals the existence of 3—4 secondary hairs on ultimate rostral segment.

Fig. 5. *Chaitophorus indicus* Ghosh, Ghosh and Raychaudhuri: Alate male. A—Posterior portion of abdomon.

5. **Diphorodon cannabis** (Passerini) (Fig. 6)
1860. *Phorodon cannabis* Passerini. Gli Afidi, 36.

Alate male: Body dark brown. Head brown, sparsely spinulose with lowly developed lateral frontal tubercles; dorsal cephalic hairs with acute apices. Antennae concolourous with head, 6-segmented: segment I with inner apex produced: flagellum gradually imbricated apicad: p.t. about 2.02 × base of segment VI; segment III with irregularly arranged 38—45 non-protuberant secondary rhinaria, segment IV and V with 14—16 and 6 such rhinaria respectively. Rostrum reaching midcoxae: u.r.s. about 1.40 × h.t. 2 bearing 4 secondary hairs. Abdominal dorsum pale with segmentally arranged spinopleural and marginal sclerotic patches: dorsal hairs similar to those on cephalic dorsum, longest one on anterior tergites being about 0.84 × b. d. III. Siphunculi long, cylindrical, with a few interconnecting striae before the apical flange, about 3.40 × cauda bearing 5 hairs. Male genitalia (Fig. 6) well-developed. F. T. C. 3, 3, 2. Wing venation normal.

Measurements of the alate male in mm: Length of body 1.80, width 0.70; antenna 1.69, antennal segments III: IV: V: VI 0.46: 0.25: 0.25: 0.09 + 0.46; u. r. s. 0.12; h.t. 2 0.08; siphunculus 0.36; cauda 0.08.

Collection data: INDIA: HIMACHAL PRADESH: Manali (c 2050 m), 27.x.79, 6 apterous viviparous ♀♀, 9 alate viviparous ♀♀, 1 alate ♂ and 1 nymph from an unidentified host plant, coll. S. K. Das.

Remark: Chowdhuri, Ghosh, Banerjee and Raychauduri (1970) reported the species from this region. Find of sexuales along with viviparae suggests the possibility of completion of holocyclic life cycle.

6. **Nippolachnus** sp. (Figs 7A—C)

Apterous oviparous female: Body pale brown, elongated oval. Head (Fig. 7A) brown, smooth without any frontal tubercle but with a median suture: dorsal cephalic hairs with acuminate apices. Antennae 6-segmented, much shorter than body: flagellum gradually imbricated apicad: p. t. about 0.66 × base of segment VI: flagellar

Fig. 6

Fig. 6. *Diphorodon cannabis* (Passerini): Alate male. Posterior portion of abdomen showing male genitalia.

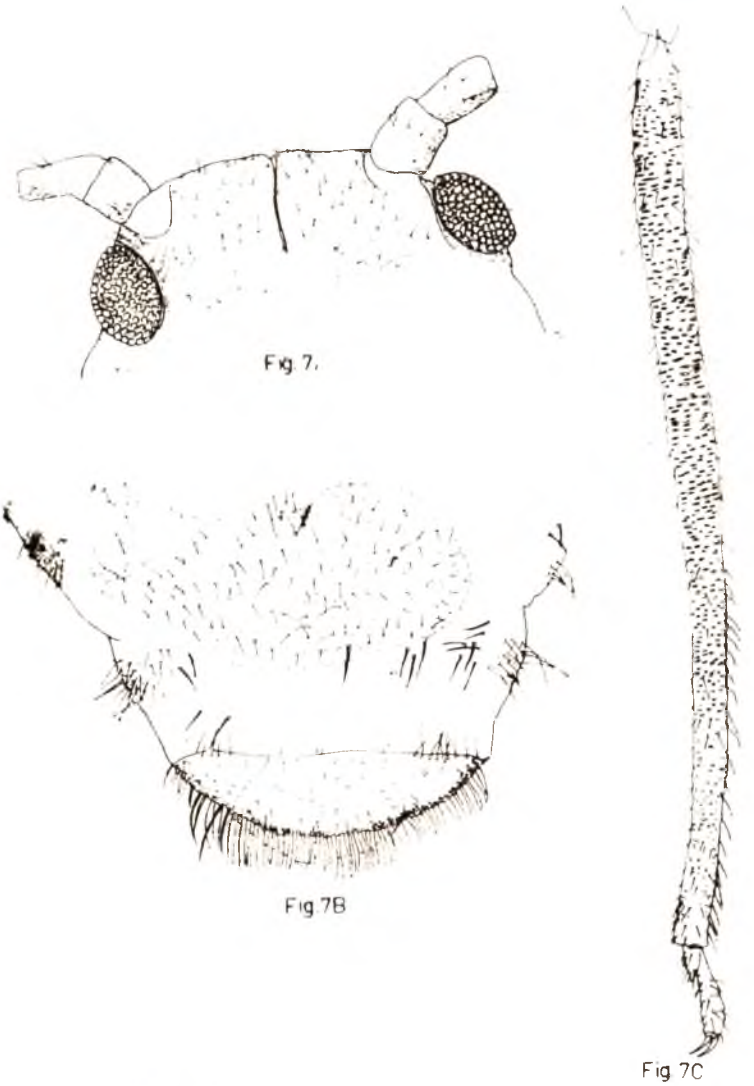


Fig. 7. *Nippolachnus* sp. : Apterous ovipara. A—Head; B—Posterior portion of abdomen; C—Hind tibiae showing pseudosensoria.

hairs with acuminate apices, longest one on segment III about 0.93 × b. d. III. Eyes multifaceted, without ocular tubercles. Rostrum extending beyond hindcoxae; u.r.s. normal, about 0.76 × h.t.2 and bears about 12 secondary hairs. Abdominal dorsum pale, with segmentally arranged sclerotic patches; dorsal hairs similar to those on cephalic dorsum, longest hair on anterior, 7th and 8th tergites about 1.20 ×, 1.0 × and 1.60 × b. d. III respectively. Siphunculi ring-like with well developed chitinized rim. Cauda helmet-shaped with numerous hairs. Subgenital plate as in Fig. 7B. Legs normal; hindtibiae swollen with numerous pseudosensoria (Fig. 7C).

Measurements of the apterous oviparous female in mm: Length of body 3.45, width 1.81; antenna 2.22, antennal segments III:

IV: V: VI 0.97: 0.42: 0.36: 0.13–0.09: u.r.s 0.18: h.t. 2. 0.24; siphuncular pore 0.10.

Collection data: INDIA: HIMACHAL PRADESH, Kufri (*c* 2633 m), 24.x.79, 1 greyish apterous oviparous ♀ and 2 nymphs from *Berberis* sp. (Berberidaceae), coll. S. K.Das.

Note: The material could not be identified upto species level because of lack of sufficient material.

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ON THE PREDACEOUS NAIAD OF *RHODOTHEMIS RUFA* (RAMBUR) (INSECTA : ODONATA)

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A fully illustrated description of the hitherto undescribed naiad of *Rhodothemis rufa* (Rambur) based on collections from two different types of habitats is presentsd here.

(*Key words* : larval description, odonate naiad, *Rhodothemis rufa*, predaceous habit)

Odonate naiads are serious pests in the aquatic habitat where they voraciously feed on fish eggs and larvae. Of late studies on the ecology of predatory insects have become extremely important. An essential prerequisite for ecological studies is the correct identification of the species involved. The present paper deals with the naiad of *Rhodothemis rufa*, a species hitherto unreported form from this country.

The material comprises twenty-three full grown naiads from Chackai canal about 4 km west of Trivandrum city collected on 8-12-1978 and eleven from a perennial pond at Kottarakkara 71 km north-east of Trivandrum collected on 17-6-1979. Temporary mounts of the various parts of the naiad were prepared in lactic acid. All the figures were drawn with the help of a camera lucida. The figures of the labium are from the oral side, and show it as flattened on the slide.

Rhodothemis rufa (Rambur) (Figs. 1—8)

1842 *Libellula rufa*, Rambur, Ins. Nevrop, p. 71.

1894 *Erythemis rufa*, Kirby, J. Linn. Soc. Zool. Vol. XXIV, p. 555.

1909 Rhodothemis Ris, Cat. Coll. Selys fasc. IX, p. 29.

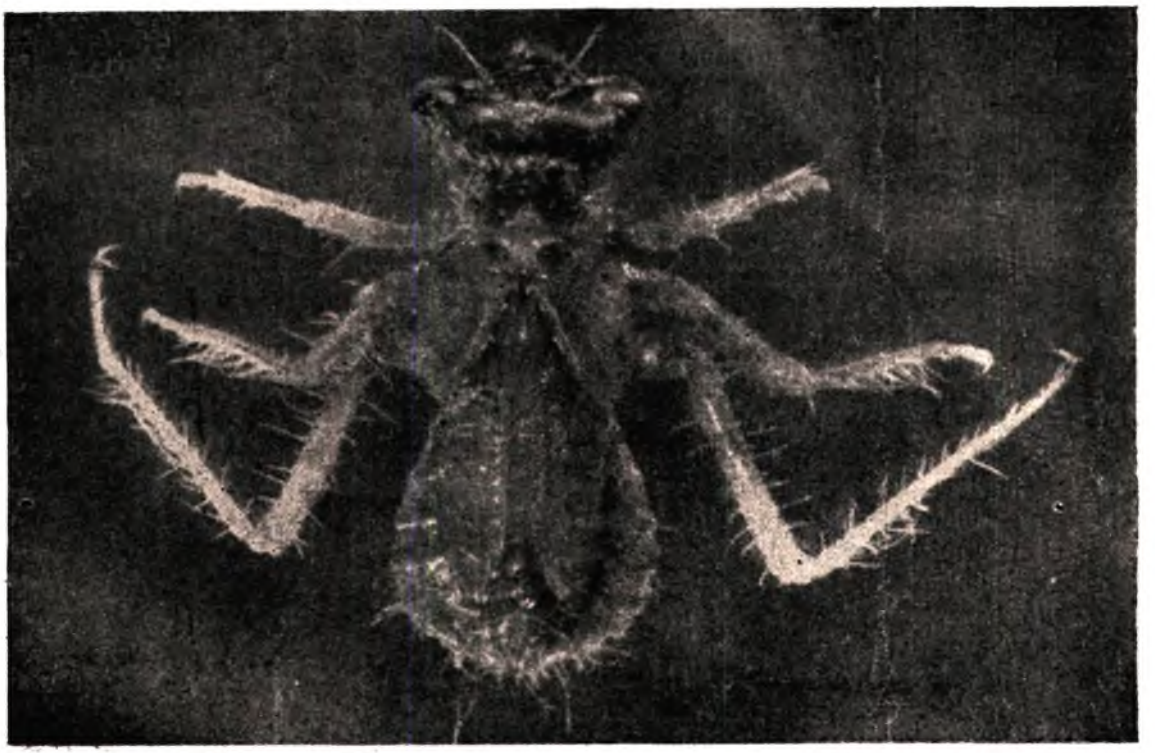
Measurements:

Body length: 19 mm; head length: 4mm, width: 5.5 mm, thorax length: 4.5 mm, width: 6 mm, abdomen length: 8.5 mm, width: 6.5 mm, anal appendages: 2 mm, wing pads: 10mm, antenna: 3.5mm, legs: 10 mm, 12 mm, 16 mm.

Length of the body varying from 18—20 mm (mean 19 mm). Maximum width 6.5 mm across the sixth abdominal segment. Body hairy.

Colour dark green or dark brown, depending on the ambient vegetation and substratum. Head with two lateral projections, hindmargin slightly convex. Eyes rounded and anterolateral. Antenna (Fig. 2) 3.5 mm, medium sized, filiform, slender and beset with hairs here and there: subequal to the distance between their bases: seven segmented; third segment longer than all the other segments and shorter than the combined length of any of the two consecutive segments. Second segment half the length of the third and the distal three segments are subequal in length.

Mask (labium) (Fig. 3) extending to base of forecoxae. Prementum almost triangular: entire margin guarded by a

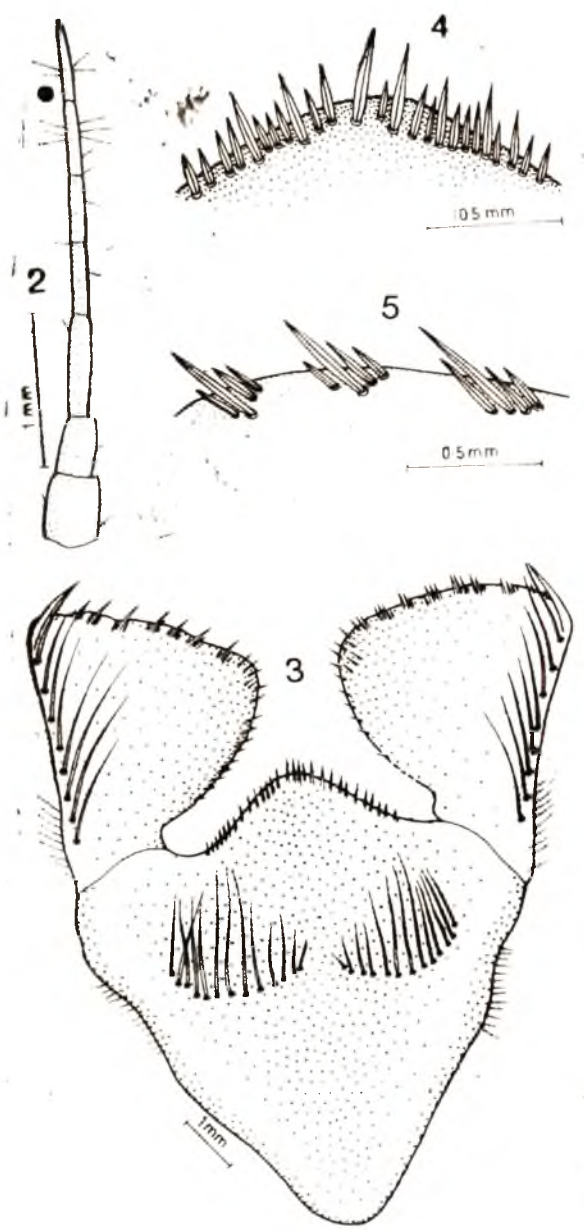


Figs. 1—3. *Rhodothemis rufa* (Rambur). 1—larva.

number of long and short spiniform setae (Fig. 4). Spiniform setae are present at the anterior portion of the prementum. Lateral margins smooth. Premental setae 13+13, rarely 14. Palpal lobe feebly crenulated each crenation bearing 3 or 4 setae (Fig. 5). Palpal setae 8 & 8, armed with small spiniform setae at the base of the palpus. Inner margin of the palpus bears small spiniform setae while the outer margins smooth. Movable hook medium sized. Mandible (Fig. 6) semitriangular with 8 teeth.

Thorax slightly broader than head, marked by two black spots at the anterior portion just in front of the origin of the wing buds. Legs are hairy, long and slender, hindfemora extending up to the posterior part of abdomen. Tibial comb (Fig. 7) consists of a few long blackish spines and simple spines. Tarsi three-segmented with a pair of claws. Posterior part of the tarsal segments and claws brownish black, the tarsi bearing a double row of long simple spines (one row black) and beset with short and long hairs here and there.

Abdomen short and semicircular, broader and longer than head and thorax and triangular in cross section. Wing buds reach the posterior part of the 7th abdominal segment. Abdomen widest at the 6th abdominal segment narrowing posteriorly. Brown markings occur mid dorsally and dorsolaterally. Posterolateral spines present on 9th segment, each crowned with



2—antenna; 3—labium; 4—enlarged view of the mid anterior margin of the prementum; 5—enlarged view of the distal margin of palpus.

4—5 small setae. Lateral spine is smaller than the anal appendages. Hairs are present on the margins of almost all the segments and those of 8th, 9th and 10th are long and regularly arranged.

Anal appendages (Fig. 8) longer than the total length of the segments 9 and 10. Paraprocts curved outwards, are longer than the epiproct which is straight and almost triangular in shape. Cerci half the length of the epiproct and shorter than half the length of the paraprocts. Both epiproct and paraprocts are beset with short hairs.

Biology:

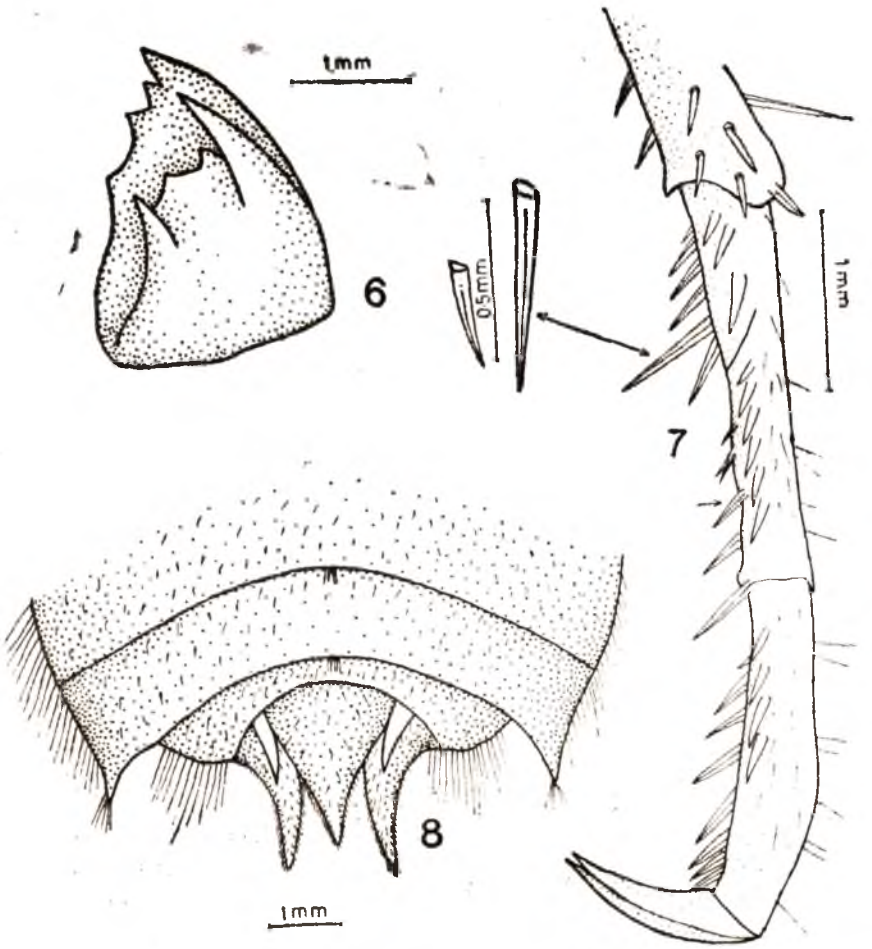
Naiads of *R. rufa* occur erratically in the habitat. Colour shows variation, specimens collected from the pond are greenish. Full-grown naiad commonly occur during the months November-December and May-June. They are primarily bottom dwellers with sluggish habits. The nymphs are gregarious, hide under aquatic plants and submerged stones or any other objects. In the laboratory they are found to be highly predaceous readily attacking mosquito larvae, tadpoles, chironomus larvae, small worms and fishfry. During the early stages they feed mainly zooplankton. Under laboratory conditions they took four months to complete their life cycle.

Distribution:

Burma, Ceylon, Java and Australia, India nov.

Remarks: Since the earlir description of the species by Snehalatha (1954) from Kerala is lacking in details, care was taken to give full illustrated description of the species on the basis of an examination of fresh material.

The main difference noticed during this study from the earlier description is regarding the number of premental and palpal setae. Specimens examined by Snehalatha had only 13 premental setae but in the present larvae 14 premental setae are noticed in some cases. Similarly in



6—mandible; 7—tibial comb; 8—anal appendages.

the present material we could notice only 8 palpal setae instead of the 9 noticed earlier.

Snehalatha had obtained her material from an artificial cement tank and the colouration of the larvae was brown. In the present study, collections were made from a natural pond and also from a brook. The larvae collected from these habitats were either brown on green.

Acknowledgement:—One of ns (K R N) is thankful to the University Grants Commission, New Delhi for the award of a research fellowship during the tenure of which the present work was carried out.

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APHIDS (HOMOPTERA : APHIDIDAE) INFESTING ROSACEOUS FRUIT PLANTS IN DARJEELING DISTRICT OF WEST BENGAL AND SIKKIM

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(Received 23 December 1979)

Quite a few rosaceous friut plants viz., apple, cherry, peach, pear and plum are grown in the Darjeeling district of West Bengal and Sikkim. These have been found to harbour as many as 15 species of aphids, of which some like *Brachycaudus helichrysi* (Kalt.), *Eriosoma laniggerum* (Haus.), *Nippolachnus piri* (Mats.) and *Tinocalloides montanus* Basu occur in pest proportion causing substantial damage. Other than these, *Aphis gossypii* (Glov.) group, *Aphis spiraecola* Patch, *Dysaphis multisetosa* Basu, *Hysteroneura setariae* (Thos.), *Myzus cerasi* (Fab.), *Myzus persicae* (Sulz.), *Pyrolachnus pyri* (Buck.), *Schyzaphis punjabipyri* (Das), *Toxoptera aurantii* Boyer de Fons. and *Thoxoptera citricidus* (Kirk.) also occur on these plants but scarsely cause any cognizable damage to them. The report deals with the key to the easy identification of the species of aphids occurring on rosaceous fruit plants and short notes on the seasonal incidence of these aphids and their pest status.

(Key words : rosaceous plants, aiphids, Darjeeling, W. Bengal, Sikkim)

Temperate fruit plants belonging to family Rosaceae that are grown extensively in Darjeeling district and Sikkim include apple *(Pyrus malus)*, cherry *(Prunus cerasus)*, peach *(Prunus persica)*, pear *(Pyrus communis)* and plum *(Prunus domestica)*. A large number of insect pests of different groups damage these plants are among aphid *Brachycaudus helichrysi* (Kalt.) has been recognised to cause serious damage to *Prunus* spp. in the Western Himalaya (Lal and Siddiqi, 1952). But no such report is available from the area of the present study and it is only Basu and Banerji (1958) who reported the occurrence of species of aphids on fruit plants belonging to Rosaceae from Darjeeling district and Ghosh (1974) reported 10 species of aphids infesting this group of plants from all over India and of these 6 occur in the area of the present study. Through a systematic study on aphids for a period of about six years from 1968 in this region it has been found that the fruit plants belonging to Rosaceae harbour as many as 15 species of aphids. An attempt has been made here to provide an easy key to the identification of the aphids occurring on these plants and short discussions have included on the seasonal incidence of various aphid species with their pest status.

Incidence of aphid species on different rosaceous fruit plants was recorded at monthly intervals from five selected localities of the region. During each observation collection of specimens was done

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and intensity of infestation and effect of infestation on the host plants were recorded. The specimens were identified by making microscopic preparations.

The data obtained through the sustained studies in the field and laboratory revealed the occurrence of 15 aphid species on rosaceous fruit plants grown in this area and they exibit variation in their seasonal occurrence. The results have been presented in the following separately for facilitating identification of species and for visualising their importance as pest and season of occurrence on different plants.

KEY TO THE IDENTIFICATION OF SPECIES: This has been based mainly on the gross morphological characters of the apterous viviparous females which are frequently found in the field.

1. Processus terminalis shorter than base of last antennal segment, secondary rhinaria either transversely oval or subanular in alatae; cauda broadly oval or basally constricted 2

   Processus terminalis at least twice as long as base of last antennal segment; secondary rhinaria mostly round; cauda elongate, never constricted or knobbed; siphunculi, cylindrical, long 6

2. Head and prothorax in apterae separate distinctly, eyes always multifaceted 3

   Head and prothorax in apterae fused, eyes in apterae 3-faceted and in alatae multifaceted with subannular secondary rhinaria on antennae; body with distinct wax glands; siphunculi poriform; cauda semilunar; fore wing in alatae with media once-branched; on apple ..... *Eriosoma lanigerum* (Hausmann)

3. Siphunculi mamiform and on hairy cones; body with numerous long hairs; first tarsal segments with more than 9 ventral hairs; secondary rhinaria in alatae large and rounded 4

   Siphunculi short, truncated cones having a hair appended near the base; cauda basally constricted, knobbed; subanal plate bilobed; tarsi usually with 3 ventral hairs on first segment; secondary rhinaria in alatae transversely oval 5

4. Eyes without any ocular tubercle; antennae in apterae usually with secondary rhinaria on segment III—IV; abdominal dorsum in alatae with dark dorsal patch; Rs of forewing slightly curved; on pear ..... *Nippolachnus piri* (Matsumura)

   Eyes with distinct ocular tubercle; antennae in apterae without secondary rhinaria; abdominal dorsum in alatae pale; Rs in forewing almost straight, on pear ..... *Pyrolachnus pyri* Buckton

5. Vertex of head with dark transverse band and connecting the compound eyes at about the middle; abdominal dorsum with irregular bands on segments 4 and 5 with various degrees of coalescence, segment 2 to 5 each with a tubercle bearing a solitary hair on the margin; siphunculi dark brown with a long hair appended to the base; apterae not known; on cherry ..... *Betacallis prunicola* Basu, Ghosh & Raychaudhuri

   Vertex without any band; abdomen with quadrangular flattish tuberculate sclerotic patches on the plural region from segment 1 to 7 and smaller marginal sclerites on segments 1 to 5; apterae known by oviparae; on cherry ..... *Tinocalloides montanus* Basu

6. Spiracles on abdominal segments 1 and 2 placed apart and usually with a small tubercle placed between them; head always smooth; antennae in apterae never with secondary rhinaria 7

   Spiracles on abdominal segments 1 and 2 palced close to each other with the sclerotic areas almost touching; antennae sometimes with secondary rhinaria in apterae ... 12

7. Hindtibiae with a row of peg like hairs along the length and with reticulation around or near the siphunculi on dorsolateral aspect of the abdomen 8

   Hindtibiae and abdomen near the siphuncular area without any peg like hairs or any pattern as above 9

8. Siphunculi less distinctly imbricated, about as long as cauda which bears 10—20 hairs;

alatae with 4—14 and 1—6 secondary rhinaria respectively on segments III and IV; on peach and pear ..... *Toxoptera aurantii* Boyer de Fonscolombe

Siphunculi distinctly imbricated, longer than cauda which bears 20—40 hairs; alatae with 10—21 and 0—4 secondary rhinaria on segments III and IV; on apple and pear ..... *Toxoptera citricidus* (Kirkildy)

9. Abdominal dorsum 7 with lateral tubercles placed posterodorsal to the spiracles 10

Abdominal dorsum 7 with lateral tubercles placed posterolateral to the spiracles 11

10. Cauda pale, elongate, constricted medially with 4 hairs; siphunculi cigar-shaped, brown imbricated about 0.75 × cauda; alatae with hindwings having only one oblique vein; on pear ..... *Hysteroneura setariae* (Thomas)

Cauda dark, blunty elongate with 6—10 hairs; siphunculi dark, cylindrical about 2.3× cauda; alatae with fore wings having media once branched, on pear ..... *Schizaphis punjabipyri* (Das)

11. Longest hair on the forefemora longer than the basal width at its junction with trochanter; cauda thumb shaped with more than 9 hairs; on peach and pear ..... *Aphis spiraecola* (Patch)

Longest hair on forefemora shorter than the width at its junction with trochanter; cauda blunt with 4—6 hairs; on apple, peach and pear ..... *Aphis gossypii* (Glover) group

12. Head smooth or slightly wrinkled, lateral frontal tubercles ill-developed and diverging; processus terminalis upto about 4× the base of the last antennal segment; cauda rather short 13

Head spinulose with well developed and parallel sided lateral frontal tubercles; cauda pale 14

13. Abdominal dorsum at least on 8th segment with paired spinal tubercles, pale and membraneous; siphunculi imbricated without preapical circumcision; cauda subpentagonal; on pear ..... *Dysaphis multisetosa* Basu

Abdominal dorsum without any tubercle, with sclerotic patches and rather long stiff hairs; siphunculi smooth with preapical circumcision; cauda short and helmet shaped; on peach, pear and plum ..... *Brachycaudus helichysi* (Kaltenbach)

14. Siphunculi cylindrical or slightly swollen on distal half about 1.5× elongate cauda; lateral frontal tubercles well developed with inner margins parallel; alatae with slightly protuberant secondary rhinaria; on pear ..... *Myzus persicae* (Sulzer)

Siphunculi always cylindrical about as long as cauda; lateral × frontal tubercles not distinctly high; alatae with normal secondary rhinaria; on peach ..... *Myzus ceraci* (Fabricius)

Seasonal history and incidence: The aphid species occurring on rosaceous fruit plants in this region exhibit variations as regards their seasonal incidence and acquisition of host plants within these plants. In the following the aphid species have been dealt with separately on these aspects.

Aphis gossypii (Glover) group

The insects are small with variable body colour which, when living, may be pale yellow, grey, pale green, dark green or black. The alatoid nymphs are normally pinkish with lateral dark wing pads on the thorax. It is highly polyphagous and cosmopolitan aphid infesting about plants belonging to different dicotyledonous plants in this region and has been found to occur throughout the year breeding mostly parthenogenetically. Rarely sexual morphs are encountered during winter months. Among rosaceous fruit plants could be recorded from *Prunus persica*, *Pyrus communis* and *Pyrus malus*. On *Prunus persica* it could be recorded only during March while on the latter two host plants it occurred from April to June and sometimes during September. The occurrence was, however, always of negligible proportion apparently without causing any direct injury to the plants. Basu and

Banerji (1958) also found this aphid to be of little importance as of pear.

Aphis spiraecola (Patch)

This is a small yellow coloured aphid comparatively bigger than *A. gossypii*. It is also a polyphagous aphid having as much as host plants belonging to different dicotyledonous plant families. It could be found to occur throughout the year on one or many of this host plants in this region though usually these become scarse during rainy season. It has been found to breed by parthenogenetic viviparity throughout the year in this region. It may however, be mentioned here that sexual morphs could also be foud during winter. Of the rosaceous fruit plants it infests *Prunus persicae* and *Pyrus communis* during May and April respectively. Usually the population is insignificant but in certain cases tender shoots of *Pyrus communis* have been found to harbour large colonies which greatly weaken the infested shoots. In general, however, its occurrence is of no economic importance.

Betacallis prunicola Basu, Ghosh and Raychaudhuri

It is a medium sized aphid of green colour having dark dorsal patches. It is known only by alate viviparous females from this region. It infests only *Prunus ceresus* and forms colonies on the young shoots during December and January. It has not been found to cause any appreciable injury to the host plant.

Brachycaudus helichrysi (Kaltenbach)

It is a small yellow to yellowish green aphid which may turn brownish when old and the apterous oviparous females are olive green in colour. This species is a highly polyphagous one and about dicotyledonous plants belonging to different families have been recorded as hosts of this aphid. It is a very notorious pest of *Prunus* spp. and of only minor importance in respect of *Pyrus* spp. The pest nature of this aphid has been recognised by Lal and Siddiqi (1954), Sharma *et al.*, (1972) and others particularly from states adjoining Western Himalaya. Recently Ghosh and Raychaudhuri (in press) have recognised this aphid as one of the serious pests of peach and plum from the Darjeeling district of West Bengal and Sikkim where they studied the biology of this aphid. It has been observed that this species occur on *Prunus* spp. from November to April while on *Pyrus* spp. it is of only transitory occurrence during December—January. Usually on *Prunus* spp. only the alatae are found in substantial numbers from late November. These are supposedly sexuparae and these give birth to nymphs that mature as apterous oviparous females which are visited by alate males from the secondary host plants (mostly *Ageratum conizoides*). These fertilised famales lay eggs on the leaf scars or branches, which hatch during early January with the bud bursting of the host plants. From fundatrix generation through fundatrigenae rapid population build up takes place, the peak being in late February to early March. This is followed by waning of the population with the formation of large number of alate migrants. The peach and plum plants become free from aphid infestation by the end of April. This population fluctuation on *Prunus* spp. follows the plant phenology indicating unsuitability of the plants for this aphid when the plants become too hard for exploitation by this aphid. It has, however, to be mentioned here that under conditions available at Kalimpong complete holocycly is not observed, i.e., when this aphid lay could resistant eggs during

December, a part of the population continues parthenogenetic viviparous reproduction on secondary host plants where the life activity during cooler months is very much slowed down.

This aphid has been found to cause serious damage to peach and plum by way of causing severe curling of new growths and shedding of flowers and young fruits. But on apple and pear it has rather no importance as pest.

Dysaphis multisetosa Basu

This is a small dark green aphid and has very frequently been found to occur on pear from the month of January when new leaves appear after winter and continues upto the month of April particularly under Kalimpong conditions. Its infestation causes curling of the leaves and moderately old leaves have been found to roll lengthwise.

Ghosh (1974) reported *Dysaphis pyri* (Boyer) from north India. Basu (1969) described *D. multisetosa* from material collected on pear in the Darjeeling district and the material that were collected and examined during the present studies conform the descriptions given by Basu (1969).

Eriosoma lanigerum (Hausmann)

It is a small aphid with flocculent wax secretion all over the body which is yellowish when wax is removed. It has recently been found to infest apple in western Sikkim. The insects are highly gregarious and form thick colonies in the branches and has been found to be quite important as pest of apple plants causing sickly growth of the plants and other deformities. This species usually appear after bud bursting of the plants in January and continues infestation almost throughout the year. The infestation reaches its peak during May-June.

Hysteroneura setariae (Thomas)

The insects are of medium size and dark brown in colour. It commonly infests graminacious plants but is known to alternate between rosaceous and graminaceous plants. In the region of present studies it has only been observed in rare occasions and in small numbers during December on *Pyrus malus*. But strikingly enougth no sexual morphs could be recorded on this plant. Therefore, it appears that though this aphid habitually infests rosaceous plants to complete holocycly such phenomenon does not probably occur in this region.

Myzus cerasi (Fabricius)

This species is medium sized and green coloured in life. This fairly polyphagous aphid could only be recorded once on *Prunus persicae* during March.

Myzus persicae (Sulzer)

This is also a medinm sized aphid of green body colour when alive. This is also a highly polyphagous apid and enjoys cosmopolitan distribution. It has been found to infest plants belonging to 46 different families of both dicotyledonous and monocotyledonous groups in this region. Its occurrence usually ranges from October to March. Among rosaceous fruit plants *Pyrus communis* has been found to be infested during March when it forms thick colonies on the young growths. In certain cases distorted growth of such shoots is noticed. Usually, however, this aphid does not occur in any heavy proportion on this plant.

Nippolachnus piri (Matsumura)

The insects are fairly big and elongate having the general body colour green with brown legs when alive. It could be recorded only on *Pyrus communis* and thus it appears to be quite host specific in this region. It has been observed to persist on this plant throughout the year. Large colonies, heavy infestation and wide-spread occurrence of this aphid are noticed from late July to early October. The population and frequency of occurrence gradually decrease from mid-October to become scarse during the period from December to April. The aphid colonises the under surface of moderately old leaves. The infested leaves gradually turn yellow and finally fall off. The young leaves when infested curl downwards. The aphids secrete honey dew profusely, the accumulation of this honey dew leaves encourages growth of sooty mould on them, which interfere with the normal photosynthesis causing debilitation of growth of the plants.

Pyrolachnus pyri (Buckton)

This is also quite big aphid of green general body colour. It could rarely be observed to infest tender branches of pear during March. Its population was never a matter of much concern.

Schizaphis punjabipuri (Das)

This small dark green aphid could be recorded from pear among the rosaceous fruit plants and has been reported to initiate pseudogalls in leaves (Ghosh, 1974). During the present study howover, such symptoms of damage could not be observed though it occurs for quite some duration i. e., December to May. Even then it has never been fond to attain pest proportion.

Tinocalloides montanus Basu

This small pale green aphid has so far been recorded from *Prunus ceresus* only and its occurrence on this plant is very much season restricted i. e., it appears during December with the appearence of new growth on the plants and continues upto March. But the population sometimes reaches appreciably high. Such high population sometimes on the under surface of the leaves the usual site of infestation, causes the leaves to curl downwards. Infestation has also been noticed on the tender shoots of the plants which also exhibit withering symptoms. The population peak of this aphid has been noticed during January-February. Sexual morphs (alate males and apterous oviparae) have been found on these plants mostly during December-January.

Toxoptera aurantii Boyer de Fonscolombe

Small black polyphagous aphid with banded antennae and pale legs has rather limited distribution being found only in the tropical and subtropical countries. As many as host plants distributed over plant families have been recorded from this region which could be found on *Prunus persica* and *Pyrus communis* among the rosaceous fruit plants. It has, however, more frequently been fond on *Pyrus communis* almost throughout the year exepting the peak rainy season i. e., July—August. In all cases the population has been found to be too low to cause any appreciable damage to the plants at the usual site of infestation, the tender shoots. On *Prunus persica* it could be recorded only during cooler month of a year.

Toxoptera citricidus Kirkildy

This small black aphid has identical distribution as *T. aurantii* and is also polyphagous but is of rather infrequent occurrence. It infests the tender twigs and may produce some distortion of such twigs. Pear and apple have been found to be

infested most by this aphid, occurrence being during the postmonsoon months, seldom also during March on pear.

It would appear that as many as 11 species of aphids infest pear plant followed by peach (5 species of aphids), apple (4 aphid species), cherry (2 aphid species) and plum (only 1 aphid species). Though 11 species have been recorded on pear only *Nippolachnus piri* is important as pest and *Dysaphis multisitosa* also causes certain amount of damage while other aphids are of no economic importance. Among 5 aphids recorded from peach *Brachycaudus helichrysi* is the only important pest of this plant and is of concern to the growers. On apple *Eriosoma lanigerum* is a well known pest. The aphids recorded on cherry do not cause any appreciable damage but plum though infested by only *Brachycaudus helichrysi* it is a very important pest causing identical damages as on peach.

Periodicity of occurrence of different aphid species on different rosaceous fruit plant species has been presented in a consolidated way in Table 1.

The aphids mostly occur during winter-spring (December—May) period of a year on all the fruit plants. This is true with regard to both number of species occurrence and the population of pest species on the plants. However, the population of pest aphid viz. *Brachycaudus helichrysi* on peach and plum, *Eriosoma lanigerum* on apple and *Nippolachnus piri* on pear have been observed during autumn-spring. Such increase in population density of the pest aphid follows the phenology of the plants i. e., when the plants put forth new shoots and foliages. With the maturiy of the shoots the population

TABLE 1. Aphid species and month(s) of their occurrence on differenent host plants.

aphid species	Month(s) of occurence on apple	cherry	peach	pear	plum
Aphis gossypi	IV, V, VI	—	III	IV-VI, IX	—
A. spiraecola	—	—	V	IV	—
Brachycaudus helichrysi	I, XII	—	I-IV,XI,XII	I, XIII	I-IV,XI,XII.
Dysaphis multisetosa	—	—	—	I-IV	—
Eriosoma lanigerum	I, XII	—	—	—	—
Betacallis prunicola	—	I, XII	—	—	—
Hysteroneura seteriae	—	—	—	XII	—
Myzus cerasi	—	—	III	—	—
M. persicae	—	—	—	III	—
Nippolachnus piri	—	—	—	I-XII	—
Pyrolachnus pyri	—	—	—	III	—
Schizaphis punjabipyri	—	—	—	I-V, XII	—
Tinocalloides montanus	—	I-III, XII	—	—	—
Toxoptera aurantii	—	—	IX	I-VI,IX-XII	—
T. citricidus	III	—	—	III	—

gradually dwindles or sometimes completely disappear during summer. The persistent species of aphids like *Eriosoma lanigerum* and *Nippolachnus piri* though exist on the host plants, their population greatly decemated during rains (June—August) and they become scarse during leaf shedding of the plants, i.e., during November—December. For *Nippolachnus piri* the abiotic depressive factors of torrential rains, while released, the population rises abruptly, which is maintained in certain cases upto October i.e., just upto the onset of winter.

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EVALUATION OF NEWER INSECTICIDES AND SOIL AMENDMENTS FOR THE CONTROL OF SWEET POTATO WEEVIL *CYLAS FORMICARIUS* FAB.

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The weevil, *Cylas formicarius* is the most serious and wide-spread pest of sweet potato crop. Field experiments conducted for its control for a period of two years using newer insecticides and soil amendments showed that treating the setts before planting and spraying the crop first after a month of planting and subsequently at triweekly interval with fenthion or fenitrothion or monocrotophos at 0.05 per cent conc. is highly effective to reduce weevil infestation leading to the increased production of quality tubers.

(Key words: newer insecticides, soil amendments, control of sweet potato weevil, *Cylas formicarius*)

INTRODUCTION

Sweet potato *(Ipomoea batatas)* is an important root crop cultivated throughout the tropical, sub-tropical and in certain temperate regions of the world. The most serious pest of this crop is *Cylas formicarius* which causes complete damage if the crop is over matured in the field. SUBRAMANIAM *et al.* (1977) reported 60—70 per cent damage in the case of severe infestation. The yield loss above 50 per cent is of common occurrence wherever the crop is continuously cultivated. Field experiments were hence conducted for the control of the pest and the results obtained are embodied in this communication.

MATERIALS AND METHODS

The experiments were conducted during 1976-78 for four seasons in RBD with four replications using a susceptible variety of sweet potato, *H*—506. Treatments were as detailed in Tables 1 and 2. Organic amendments and granular insecticide were applied as pre-plant treatment as well as post plant treatment after 45 days. The emulsifiable concentrates were sprayed first after a month of planting and subsequently at triweekly intervals. The setts were also dipped in insecticides before planting. Altogether four sprayings were done. The efficacy of different treatments was assessed on the basis of the quantity of uninfested tubers obtained and also on the basis of yield loss due to weevil damage.

RESULTS AND DISCUSSION

It could be seen from the data in Table 1 that the percentages of weevil damage in different treatments ranged from 4.92 to 32.60 in the first season recording minimum in fenitrothion and maximum in neem cake treatment. The treatment monocrotophos also produced lower damage. Neem cake and neem cake + furadan treatments were found statistically on par with control. The rest of the chemicals were almost on par and significantly inferior to fenitrothion and monocrotophos. In the second season also the treatments fenitrothion and monocrotophos were on par and significantly superior to all other treatments. These treatments gave the yield of uninfested tubers to the extent of 12.40 and 11.97

TABLE 1. The effect of different treatments on the incidence of *C. formicarius* and on the yield of sweet potato (76—77).

S. No.	Treatments and concentration	Seasons					
		March—June 1976			October—Janudry 76—77		
		Good tuber ton/ha	Percent damage		Good tuber ton/ha	Percent damage	
1.	Neem cake @ 500 kg/ha	0.76	32.60	(34.80)*	8.70	28.65	(32.30)
2.	Furadan @ 1 kg a i/ha	1.49	24.80	(28.00)	8.77	18.62	(25.55)
3.	Neem cake + Furadan @ 500 kg/ha + 1 kg a i/ha	1.09	30.60	(33.52)	9.86	24.79	(29.80)
4.	Fenitrothion @ 0.05%	1.99	4.92	(12.75)	12.40	2.80	(9.50)
5.	Formothion @ 0.05%	1.78	15.40	(23.05)	10.50	17.11	(24.37)
6.	Nuvan @ 0 05%	1.47	20.87	(27.05)	8.05	23.95	(29.10)
7.	Monocrotophos @ 0.05%	1.54	6.32	(14.45)	11.97	4.69	(12.47)
8.	Carbaryl @ 0.1%	1.54	17.17	(23.40)	8.00	21.50	(27.55)
9.	Dicrotophos @ 0.05%	0.91	18.32	(25.00)	10.79	8.54	(16.87)
10.	Control	0.64	35.25	(36.32)	4.33	52.74	(46.57)
		C. D. at 5% = 7.54				C. D. at 1% = 5.80	

* Figures in parantheses are transformed values.

Note: Neem cake and Furadan were applied as pre-plant and post plant treatments at the above rate. In neem cake+furadan treatment, cake was applied at pre-planting stage and Furadan at tuber forming stage.

TABLE 2. The effect of difierent treatments on the incidence of *C. formicarius* and on the yield of sweet potato (77—78).

S. No	Treatments and concentrations	Seasons					
		June—September 77			October—January 1977-78		
		Good tubers ton/ha	Percent damage		Good tubers ton/ha	Percent damage	
1.	Lime @ 2 tons/ha	3.88	59.11	(50.85)*	3.14	47.19	(43.39)*
2.	Cashewnut shell @ 2 tons/ha	6.58	38.80	(37.58)	3.90	48.20	(43.96)
3.	Cashewnut cake @ 2 tons/ha	6.55	37.63	(37.92)	3.64	42.85	(40.82)
4.	Formothion @ 0.05%	5.92	51.23	(46.77)	4.84	42.70	(40.22)
5.	Malathion @ 0.05%	4.84	58.55	(49.67)	3.84	45.55	(42.44)
6.	Thiometon @ 0.05%	5.28	62.94	(52.55)	4.70	36.77	(37.38)
7.	Methyldemeton @ 0.05%	2.58	63.36	(52.55)	3.84	45.25	(41.82)
8.	Fenitrothion @ 0.05%	11.33	13.77	(21.84)	6.66	10.36	(18.78)
9.	Fenthion @ 0.05%	13.77	8.35	(16.35)	8.06	6.44	(14.63)
10.	Control	3.14	68.44	(55.90)	1.94	67.26	(55.57)
	CD at 5% for transformed values	—	9.03		—	6.15	

* Figures in parantheses are trsnsformed values.

Note: Lime was applied as pre-plant treatment, while cashewnut shell and cake were applied both as pre-plant and post plant treatments

ton ha respectively as against only 4.33 ton/ha in the control.

The data presented in Table 2 show the efficacy of another set of treatments during 1977—78. It could be seen from Table 2 that the chemicals fenthion and fenitrothion were statistically on par and significantly superior to all other treatments having produced lower weevil damage and greater yields of good tubers. These treatments produced the yields of uninfested tubers to the tune of 13.77 ton/ha and 11.33 ton/ha respectively as against only 3.14 ton/ha in the control with 68.44 per cent weevil damage. In the second season also fenthion and fenitrothion showed their superiority over others. The yields of uninfested tubers in the respective treatments of fenthion and fenitrothion were in the order of 8.06 ton/ha and 6.66 ton/ha against only 1.94 ton/ha in the control, while in other treatments the yields ranged from 3.14 to 4.84 ton/ha only.

The data presented in Table 3 show the mean yields of effective treatments and the control (mean of four seasons) along with the costs for the treatments and the benefit over the control. The cost benefit ratio was maximum in fenthion followed by fenitrothion and minimum in monocrotophos.

Thus the results of the field experiments conducted in four seasons during two years have shown that the treatments fenthion, fenitrothion and monocrotophos are highly effective to reduce weevil damage on sweet potato and thereby to increase the production of healthy tubers. PILLAI & MAGOON (1969) had earlier established the efficacy of fenitrothion for the control of weevil. Hence fenitrothin was used as the standard chemical to compare other treatments in all the trials. The results of the present experiments showed that fenitrothion, fenthion and monocrotophos are all statistically on par. SUBRAMANIAM *et al.* (1973) reported that spraying fenthion

TABLE 3. Mean yield and the cost inolved in the treatments with benefit ratio in comparison to control.

Treatments	Mean quantity of good tubers (t/ha)	Market value Rs	Qty. of chemicals for 4 sprayings. (per/ha)	Cost of chemical (Rs)	Labour charge for spraying (Rs)	Total for chemical & labour (Rs)	Net income	Benefit ratio with control
Monocrotophos (Nuvacron)	6.98	2792	5 lit.	825	240	1065	1727	1:1.72
Fenitrothion (Sumithion)	8.10	3240	4 lit.	288	240	528	2712	1:2.70
Fenthion (Lebaycid)	10.10	4040	2 lit.	236	240	476	3564	1:3.55
Control	2.51	1004	—	—	—	—	1004	—

Note: Cost of cultivation that are common for control and treatments are not taken into account, only the additional expenditures involved for treatments are considered here. Market value of the produce is calculated @ Rs. 400/- per ton of tubers.

0.1% or carbaryl 0.1% at triweekly intervals is effective against the weevil. The present study shows that fenthion at a lower concentration (0.05%) is sufficient for the control of the weevil and carbaryl is ineffective.

After establishing the efficacy of fenthion, fenitrothion and monocrotophos for the control of the weevil, the tubers obtained from these treatments were got analysed at CFTRI, Mysore to find out whether any toxic residues are left behind that may become hazardous for consumption. The results of the analysis showed that the residue is nil in the case of monocrotophos and 0.09 ppm in fenitrothion and 0.07 ppm in fenthion which are far below the tolerance limit, hence can be safely used. It is, therefore, confirmed and concluded that spraying sweet potato crop first after a month of planting and subsequently at triweekly intervals with fenthion or fenitrothion or monocrotophos at 0.05 percent concentration of active ingredient is highly effective to reduce weevil infestation leading to the increased production of uninfested tubers. The benefit ratios of the control and treatments have shown that the most effective treatment is fenthion followed by fenitrothion as these treatments respectively give 3.55 and 2.70 times more profit than the untreated control.

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CHEMICAL CONTROL OF THE APHID, *APHIS AFFINIS* DEL GUERCIO (HOMOPTERA: APHIDIDAE) - A PEST OF JAPANESE MINT, *MENTHA ARVENSIS* LINN. IN THE PUNJAB

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Ten insecticides (5 systemic and 5 contact), viz., formothion, thiometon, dimethoate, phosphamidon, oxydemeton methyl, endosulfan, malathion, fenitrothion, phenthoate and phosalone at 0.025 and 0.05 per cent were tested under screen-house conditions on the potted plants. It was found that thiometon, dimethoate, phosphamidon and oxydemeton methyl gave the maximum mortality under green-house conditions. These chemicals were further tested under field conditions at the same concentrations. It was observed that thiometon and oxydemeton methyl gave the maximum mortality at 0.05 per cent concentration and were regarded as the most effective chemicals.

(Key words: chemical control, *Aphis affinis*, Japanese mint, *Mentha arvensis*)

INTRODUCTION

India is far from self-sufficiency in pharmaceutical products. Drugs worth millions of rupees have to be imported every year. To enable the expanding pharmaceutical industry in the country, it is necessary to get the maximum output of *Mentha* oil which warrants careful attention to this crop. More than 95 species of insects have been recorded on this crop by different scientists (ANONYMOUS, 1977; FURSOVA, 1972; GOULD, 1960; SANDHU *et al.*, 1975).

In the Punjab the aphid, *Aphis affinis* del Guercio was first observed on Japanese mint in 1977 (ANONYMOUS, 1977). The aphid colonies suck the cell sap from the leaves and stunted plant growth, thus yield of the crop is reduced. In addition to direct losses to the crop, the aphid also acts as vector of cucumber mosaic virus (HEINZE, 1959). Keeping in view the importance of the pest, a chemical control trial using some contact and systemic insecticides was conducted and the results are presented in this paper.

MATERIALS AND METHODS

Experiments to test the effectiveness of ten insecticides, viz., dimethoate, endosulfan, fenitrothion, formothion, malathion, oxydemeton methyl, phenthoate, phosalone, phosphamidon and thiometon with different concentrations were conducted against the aphid infestation on the potted plants and also in the field.

Experiment on potted plants: The above mentioned systemic and contact insecticides (each at 0.025 and 0.05 per cent) were tested with a view to find out their effective concentration. There were 4 replications, one plant represented a replication. The insecticide was sprayed uniformly on each plant with the help of poly-sprayer in the following two ways:

i) Spraying the plants after infesting them with the aphids. ii) Spraying the plants and then infesting them with the aphids

In the former case the number of aphids was counted on each plant before spraying. Whereas in the latter case 25 aphids per plant were confined 1, 2, 7, 14 and 21 days after spraiyng and aphid mortality was recorded 24 hours thereafter. The mean per cent mortality in a trearment was calculated for the purpose of statistical analysis.

Experiment in the field: The promising pesticides, viz., dimethoate, oxydemeton methyl, phosphamidon and thiometon (each at 0.025 and 0.05 per cent) were tried in the field. The trial was laid out in a randomized block design with five replications and plot size was 10×3 metres. The insecticides were applied with the help of polysprayer. From each plot 5 plants selected at random were marked with aluminium tags for all the observations. Pre-treatment population of the aphid was recorded before spraying. The post-spray observations were made after 1, 2, 7, 14 and 21 days. The per cent mortality was calculated and the data analysed statistically.

RESULTS AND DISCUSSION

Experiment on potted plants: The data of the experiment in which aphids were released after spraying the systemic insecticides are presented in Table 1. It can be

TABLE 1. Comparative efficacy of systemic insectcides against *A. affinis* when the aphids were released after spraying the potted plants.

Insecticides tested		*Mean per cent reduction in population after indicated number of days				
Insecticide	Concentration	1	2	7	14	21
Formothion	0.025	85.0 (67.25)^{c}	83.0 (65.68)^{d}	76.0 (60.68)^{c}	67.0 (54.94)^{e}	55.0 (47.87)^{d}
Formothion	0.05	100 0 (90.00)^{a}	100.0 (90.00)^{a}	98.1 (82.00)^{a}	91.1 (72 62)^{b}	80.0 (63.46)^{b}
Thiometon	0.025	96.1 (78.58)^{b}	96.3 (78.90)^{b}	89.0 (70.46)^{b}	75.0 (60.04)^{d}	60.0 (50.77)^{d}
Thiometon	0.05	100 0 (90.00)^{a}	100.0 (90.00)^{a}	98.5 (82.98)^{a}	91.1 (72.62)^{b}	80.0 (63.46)^{b}
Dimethoate	0.025	85.2 (66.35)^{c}	83.1 (63.71)^{d}	76.0 (60.67)^{c}	67.5 (55.26)^{e}	55.0 (47.87)^{d}
Dimethoate	0.05	100.0 (90.00)^{a}	100.0 (90.00)^{a}	98.5 (83.5)^{a}	91.1 (72.62)^{b}	80.0 (63.46)^{b}
Phosphamidon	0.025	95.4 (77.66)^{b}	95.2 (77.36)^{bc}	90.3 (71.84)^{b}	81.0 (64.17)^{c}	70.1 (56.87)^{c}
Phosphamidon	0.05	100.0 (90.00)^{a}	100.0 (90.00)^{a}	99.3 (85.10)^{a}	96.5 (79.23)^{a}	90.6 (72.12)^{a}
Oxydemeton methyl	0.025	95.4 (77.66)^{b}	94.0 (75.84)^{c}	86.1 (68.10)^{b}	72.0 (58.06)^{de}	54.1 (47.37)^{c}
Oxydemeton methyl	0.05	100.0 (90.00)^{a}	100.0 (90.00)^{a}	99.3 (85.10)^{a}	93.0 (74.67)^{b}	86.1 (68.14)^{ab}
Control**	—	—	—	—	—	—
(S E)		(1.20)	(0.86)	(1.64)	(0.05)	(1.77)

* Mean of four replications. Figures followed by same alphabets in a column do not differ significantly as per Duncan's multiple-range test. Figures in parentheses are the arcsin $\sqrt{\text{percentage}}$ values.

** There was no aphid mortality in the control.

TABLE 2. Comparative efficacy of systemic insecticides against *A. affinis*, spraying done on infested plants.

Insecticides tested		Pre-treatment population	*Mean per cent reduction in population after indicated number of days				
Insecticide	Concentration		1	2	7	14	21
Formothion	0.025	145	46.8 (43.17)^{d}	71.0 (57.42)^{d}	63.0 (52.53)^{d}	52.0 (46.15)^{f}	39.9 (39.15)^{f}
Formothion	0.05	130	88.4 (70.90)^{c}	88.0 (69.74)^{c}	81.1 (64.25)^{e}	69.2 (56.32)^{cd}	57.0 (49.02)^{d}
Thiometon	0.025	133	100.0 (90.00)^{a}	100.0 (90.00)^{a}	98.1 (82.00)^{a}	88.1 (69.80)^{b}	72.0 (58.06)^{c}
Thiometon	0.05	155	100.0 (90.00)^{a}	100.0 (90.00)^{a}	98.1 (82.00)^{a}	91.0 (72.89)^{ab}	79.4 (62.99)^{b}
Dimethoate	0.025	127	89.0 (70.64)^{c}	89.0 (70.64)^{c}	80.1 (63.49)^{c}	66.0 (54.36)^{d}	50.00 (44.99)^{b}
Dimethoate	0.05	118	97.2 (80.24)^{b}	97.2 (80.40)^{b}	94.4 (76.26)^{b}	89.0 (70.67)^{b}	80.0 (63.46)^{b}
Phosphamidon	0.025	167	100.00 (90.00)^{a}	100.00 (90.00)^{a}	94.5 (76.47)^{b}	71.1 (59.38)^{c}	47.0 (43.28)^{c}
Phosphamidon	0.05	159	100.0 (90.00)^{a}	100.0 (90.00)^{a}	98.1 (82.00)^{a}	94.7 (76.63)^{a}	87.6 (69.40)^{a}
Oxydemeton methyl	0.025	153	100.0 (90.00)^{a}	100.0 (90.00)^{a}	98.1 (82.00)^{a}	91.2 (72.76)^{ab}	79.3 (62.95)^{b}
Oxydemeton methyl	0.05	165	100.0 (90.00)^{a}	100.0 (90.00)^{a}	98.6 (83.30)^{a}	90.6 (76.57)^{a}	85.2 (67.34)^{a}
Control**							
(S E)	NS	NS	(0.94)	(0.54)	(1.52)	(1.55)	(1.00)

* Mean of the four replications.
— Figures followed by the same alphabets in a colum do not differ significantly as per Duncan's multiple-range test.
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** There was no aphid mortality in the control.

seen that all the insecticides at 0.05 per cent concentration gave 100 per cent mortality of the aphid one and two days after spraying. One day after spraying thiometon, phosphamidon and oxydemeton methyl at 0.025 per cent sprays proved significantly better than formothion and dimethoate. Formothion, thiometon, dimethoate, phosphamidon and oxydemeton methyl at 0.05 per cent concentration were equally and significantly higher than at 0.025 per cent concentration after 1, 2 and 7 days of spraying.

Fourteen days after spraying phosphamidon 0.05 per cent proved most effective and gave 96.5 per cent mortality of the aphid. Phosphamidon 0.05 per cent retained its superiority to all other treatments except oxydemeton methyl 0.05 per cent even 21 days after spraying.

The results of second experiment are presented in Table 2. It revealed that one day after spray, thiometon, phosphamidon

and oxydemeton methyl at both the concentrations gave 100 per cent mortality of aphids and were significantly better than the remaining treatments. Formothion 0.025 per cent proved more or less ineffective as it gave only 46.8 per cent mortality. Similar trends were observed 2 and 7 days after spraying.

Fourteen days after spraying, thiometon 0.05 per cent, phosphamidon 0.05 per cent and oxydemeton methyl 0.025 and 0.05 per cent showed non-significant differences. The other treatments showed a much lower persistance. Twentyone days after spraying only phosphamidon and oxydemeton methyl 0.05 per cent were significantly more promising than rest of the treatments.

Considering the overall effectiveness it may be inferred that all the insecticides at higher concentration (0.05 per cent) were better than control.

TABLE 3. Comparative efficacy of contact insecticides against *A. affinis*, aphids released on sprayed plants.

Insecticides tested		*Mean per cent reduction in population after indicated number of days				
Insecticide	Concentration	1	2	7	14	21
Endosulfan	0.025	90.1 (71.68)^{c}	85.2 (68.19)^{c}	71.5 (57.74)^{d}	57.2 (42.12)^{f}	18.8 (25.67)^{f}
Endosulfan	0.05	95.2 (77.36)^{b}	94.3 (76.21)^{b}	83.1 (65.75)^{c}	61.0 (51.37)^{e}	35.0 (36.26)^{d}
Malathion	0.025	100.0 (90.00)^{a}	100.0 (90.00)^{a}	87.1 (80.25)^{ab}	81.0 (64.17)^{c}	60.0 (50.77)^{c}
Malathion	0.05	100.0 (90.00)^{a}	100.0 (90.00)^{a}	97.2 (80.30)^{ab}	86.1 (68.11)^{c}	70.1 (56.83)^{b}
Fenitrothion	0.025	74.6 (59.72)^{d}	68.0 (55.58)^{d}	57.0 (49.02)^{e}	44.0 (41.55)^{f}	2.99 (33.17)^{e}
Fenitrothion	0.05	90.2 (71.71)^{c}	89.0 (70.65)^{c}	78.0 (62.06)^{cd}	60.0 (50.77)^{e}	40.0 (30.21)^{d}
Phenthoate	0.025	100 0 (90.00)^{a}	100.0 (90.00)^{a}	93.2 (74.86)^{b}	68.0 (55.55)^{d}	35.0 (36.26)^{d}
Phenthoate	0.05	100.0 (90.00)^{a}	100.0 (90.00)^{a}	99.3 (85.10)^{a}	93.1 (74.72)^{b}	85.0 (67.23)^{a}
Phosalone	0.025	100.0 (90.00)^{a}	100.0 (90.00)^{a}	98.1 (82.00)^{a}	85.8 (67.88)^{c}	70.0 (56.81)^{b}
Phosalone	0.05	100.0 (90.00)^{a}	100.0 (90.00)^{a}	99.3 (85.10)^{a}	96.2 (78.74)^{a}	90.0 (71.59)^{a}
Control**						
(S E)		0.78	0.98	1.69	0.99	1.69

* Mean of four replications.

— Figures followed by same alphabets in a column do not differ significantly as per Duncan's multiple-range test.

— Figures in parenthese are the arcsine $\sqrt{\text{percentage}}$ values.

** There was no mortality in the control.

The data of the third experiment presented in Table 3 revealed that malathion, phenthoate and phosalone at both the concentrations (0.025 and 0.05 per cent) gave 100 per cent kill of the aphid 1 and 2 days after spraying. However, 7 days after spraying phosalone and malathion (0.025 and 0.05 per cent) and phenthoate (0.05 per cent) proved equally effective. Endosulfan and fenitrothion (0.025 per cent) proved least effective.

Fourteen days after spraying phosalone 0.05 per cent gave significantly higher kill (96.2 per cent) of the aphid and retained its persistance up to 21 days and proved better than all treatments except phenthoate 0.05 per cent.

The results of 4th experiment are presented in table 4 which indicated that endosulfan (0.025 and 0.05 per cent), malathion and phosalone (0.05 per cent) gave 100 per cent mortality 1 and 2-days after spraying. Seven days after spraying phenthoate 0.05 per cent registered the highest

TABLE 4. Comparative efficacy of contact insecticides against *A. affinis*, when the spraying was done on infested plants.

Insecticides tested		Pre-treatment population	Mean* per cent reduction in population after indicated number of days				
Insecticide	Concentration		1	2	7	14	21
Endosulfan	0.025	148	100.0 (90.00)^{a}	100.0 (90.90)^{a}	96.8 (79.63)^{a}	84.1 (66.49)^{abc}	66.2 (54.43)^{bc}
Endosulfan	0.05	150	100.0 (90.00)^{a}	100.0 (90.00)^{a}	97.0 (80.80)^{ab}	86.2 (68.19)^{ab}	69 0 (56.17)^{ab}
Malathion	0.025	130	96.2 (78.65)^{b}	94.5 (76.47)^{be}	90.1 (71.64)^{dc}	75.3 (60.20)^{d}	60.0 (50.77)^{cd}
Malathion	0.05	128	100.0 (90.00)^{a}	100.0 (90.00)^{a}	97.0 (80.80)^{ab}	86.0 (68.03)^{ab}	70.1 (56.80)^{ab}
Fenitrothion	0.025	128	95.0 (74.99)^{c}	91.2 (72.69)^{c}	85.0 (67.78)^{e}	61.0 (51.35)^{f}	40.0 (39.22)^{e}
Fenitrothion	0.05	135	100.0 (90.00)^{a}	100.0 (90.00)^{a}	94.1 (75.97)^{bcd}	72.0 (58.05)^{de}	45.0 (42.11)^{e}
Phenthoate	0.025	127	88.3 (71.00)^{d}	86.3 (68.27^{d}	78.0 (62.10)^{f}	68.0 (56.58)^{e}	55.5 (48.19)^{d}
Phenthoate	0.05	130	100.0 (90.00)^{a}	100.0 (90.00)^{a}	98.1 (82.00)^{a}	89.3 (70.87)^{a}	75.0 (60.02)^{a}
Pho salone	0.025	110	97.2 (80.34)^{b}	96.5 (79.28)^{b}	91.3 (72.87)^{cde}	78.0 (62.00)^{cd}	65.0 (53.74)^{bc}
Phosalone	0.05	176	100.0 (90.00)^{a}	100.0 (90.00)^{a}	96.1 (78.65)^{abc}	83.2 (65.79)^{bc}	72.7 (58.50)^{ab}
Control**							
(S E)		N S	1.11	1.45	1.79	1.46	1.22

* Mean of for replications.
— Figures followed by same alphabets in a column do not differ significantly as per Duncan's multiple-range test.
— Figures in parentheses are the arcsine $\sqrt{\text{percentage}}$ values.
** There was no aphid mortality in the control.

TABLE 5. Comparative efficacy of promising insecticides under field conditions.

Insecticides tested		Pre-treatment population	Actual toxicant per acre (ml)	*Mean per cent reduction in population after indicated number of days				
Insecticide	Concentration			1	2	7	14	21
Thiometon	0.025	119	75.0	70.8 (57.29)^{c}	70.8 (57.29)^{e}	67.5 (55.24)^{g}	63.9 (53.06)^{d}	52.6 (46.49)^{d}
Thiometon	0.05	135	150.0	96.8 (79.69)^{a}	37.8 (81.99)^{a}	92.3 (73.92)^{ab}	86.8 (68.71)^{a}	66.0 (54.35)^{ab}
Dimethoate	0.025	147	75.0	70.6 (57.17)^{c}	84.4 (66.71)^{cd}	80.0 (63.46)^{ef}	70.2 (56.91)^{ab}	57.2 (49.14)^{cd}
Dimethoate	0.05	160	150.0	87.0 (68.90)^{b}	92.2 (73.74)^{b}	89.9 (71.76)^{bc}	80.7 (63.94)^{ab}	66.0 (54.35)^{ab}
Phosphamidon	0.025	151	90.0	78.0 (62.00)^{c}	81.4 (64.66)^{d}	76.7 (61.17)^{f}	59.3 (50.33)^{e}	37.8 (37.96)^{e}
Phosphamidon	0.05	129	180.0	97.2 (80.33)^{a}	98.0 (81.86)^{a}	95.2 (77.32)^{a}	79.8 (63.31)^{bc}	62.3 (52.10)^{bc}
Oxydemeton methyl	0.025	131	62.5	88.0 (70.46)^{b}	89.6 (71.23)^{bc}	84.3 (66.67)^{de}	74.7 (59.77)^{bc}	69.10 (56.19)^{a}
Oxydemeton methyl	0.05	155	125.0	96.4 (79.02)^{a}	92.5 (74.11)^{b}	87.3 (69.14)^{cd}	77.9 (61.94)^{b}	79.8 (57.30)^{a}
Control**								
(S E)		N S	—	1.60	1.60	1.38	2.91	2.88

* Mean of five replications.

— Figures followed by same alphabets in a column do not differ significantly as per Duncan's multiple-range test.

— Figures in parentheses are the arcsine $\sqrt{\text{percentage}}$ values.

** There was no aphid mortality in the control.

aphid mortality (98.1 per cent) which was significantly better than malathion, phenthoate and phosalone (0.025 per cent) and fenitrothion (0.025 and 0.05 per cent).

Fourteen days after spraying phenthoate, malathion (0.05 per cent) and endosulfan (0.025 and 0.05 per cent) were better than all other treatments except phosalone (0.05 per cent) which was at par with phosalone (0.025 per cent). Twenty one days after sparying only phenthoate (0.05 per cent) gave 75.0 per cent mortality which was, however, at par with that recorded in endosulfan, malathion and phosalone (0.05 per cent). The remaining treatments gave lower kill of the aphid.

On the basis of above experiments it may be concluded that endosulfan, malathion, fenitrothion, phenthoate and phosalone 0.05 per cent concentration proved very effective against the aphid,

Experiments in the field: The field experiment could be arranged only with the promising systemic insecticides. The data are presented in Table 5. It may be seen that all the insecticides proved significantly better than the control up to 21 days

after spraying. Thiometon, phosphamidon and oxydemeton methyl at 0.05 per cent resultad 96.4 to 97.2 per cent reduction in population and proved better than the other treatments. Two days after spraying the highest reduction in population (98.0 per cent) was registered by phosphamidon (0.05 per cent) which was, however, at par with that of thiometon (0.05 per cent). Phosphamidon (0.05 per cent) retained its effectiveness up to 7 days after spraying, however, it showed non-significant differences with thiometon (0.05 per cent) which in turn was at par with dimethoate (0.05 per cent).

Fourteen days after spraying the highest reduction in population was observed in case of thiometon (0.05 per cent). It was at par with that recorded in case of dimethoate (0.05 per cent) which showed non-significant differences with that of oxydemeton methyl (0.025 per cent). Twenty one days after spraying the kill was more or less the same in case of oxydemeton methyl (0.025 and 0.05 per cent), thiometon and dimethoate (0.05 per cent). From this trial it may be concluded that thiometon (0.05 per cent) and oxydemeton methyl (0.025 & 0.05 per cent) proved most effective until 21 days.

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